

BOOK OF ABSTRACTS



**Feed2021** virtual conference!

International Feed Conference

Vienna, Austria | June 23-24, 2021

PRESENT AND FUTURE CHALLENGES



UNIVERSITÀ DEGLI STUDI DI MILANO



Virtual Conference



# Feed2021 virtual conference!

## International Feed Conference

Vienna, Austria | June 23-24, 2021

Feed2021 is the seventh in a series of conferences initiated by the European Union's leading reference laboratories and research institutions in animal feed.

### Present and Future Challenges

As seen in 2020 under the aspect of the COVID-19 pandemic, the availability of sufficient and safe feedingstuff is still a key challenge in modern agriculture. While the topic of undesirable substances in feed remains a major issue, the globalisation of the feed business has further reinforced the need for efficient tools for traceability of feed ingredients. Moreover, the constantly increasing demand for food from animal origin, along with limited resources triggers the need for evaluating new sources of feed ingredients such as insects and efficient feed production. Additionally, the impact of climate change on feed production should be also taken into account. Keeping the feed safe and sustainable therefore requires a multidisciplinary approach, bringing together all stakeholders, including the industry.

The Organization Committee of this International Feed Conference tried to cover the current interesting areas for animal feed, which will be presented within the following five sessions:

- **Feed Safety**
- **Feed Fraud and Feed Authenticity**
- **Natural Toxins and Impact of Climate Change**
- **Sustainability and Circular Economy in the Area of Feed**
- **Impact of Feed on Animal Health and Welfare**

In today's world, sharing of scientific knowledge, research findings, laboratory methods and strategies within the scientific community has become a necessity. The aim of this conference is to bring scientists, researchers, laboratory personnel, policy-makers from governmental and non-governmental organizations and people from industry on a single platform where they can share their knowledge, scientific experiences and experiments on subjects crucial to animal feed. With the participation of international experts, we hope that productive discussions will stimulate new creative ideas to translate new discoveries into better practice and application.

## Meeting the challenges in feed production with research and innovative projects



PREFACE BY ELISABETH KÖSTINGER  
AUSTRIAN FEDERAL MINISTER FOR AGRICULTURE  
REGIONS AND TOURISM

It is indispensable for a smoothly functioning agriculture that a sufficient quantity of safe and high-quality feedstuffs is available on a long-term basis. In order to reach that goal, stakeholders from various fields must cooperate. Innovative ideas and new scientific findings play an important role for a sustainable and future-proof feed production.

### Sustainably safeguarding feed production

As all other fields of agriculture, feed production must be oriented according to the principle of sustainability. The Sustainable Development Goals of the UN constitute an important compass in this respect. Apart from safeguarding the subsistence and competitiveness, sustainable food production and the preservation of species-rich cultivated landscapes constitute major concerns of the Austrian agricultural policy. Therefore, the Federal Ministry of Agriculture, Regions and Tourism welcomes in principle the European Future Strategies "Biodiversity 2030" and "From Farm to Fork". By 2030 the use as well as the risk of chemical plant protection products shall be reduced by a total of 50 %.

As regards the issue of plant protection we, in Austria, are fully in line with the motto "As little as possible as much as necessary". Therefore we are already implementing numerous measures aiming at an environmentally sound and sustainable production as well as at the reduction of chemical plant protection products. With the Austrian Agri-environmental Programme (ÖPUL) Austria's programme for the promotion of an agriculture, which is environmentally compatible, extensive and protective of natural habitats, we are working on guaranteeing food security on the long run, promoting sustainable water management and sustainable types of production, and protecting terrestrial ecosystems. Moreover, the further development of our plant protection alert service plays an important role in this respect.

### A differentiated implementation of the EU Future Strategies is necessary

Within the Framework of the National Strategic Plan of the Common Agricultural Policy of the EU we will make a contribution by means of appropriate measures in order to ensure that these ambitious goals can be reached. However, what is particularly important to us in this respect, is that the individual goals and measures are looked at closely and analysed with a view to their feasibility and their overall effects - also taking into consideration the farms and the food chain. Economic incentives are indispensable for a development towards more sustainability and environmental protection. Levels reached or advance achievements accomplished by individual Member States must be considered in any case. In Austria, for example, 26 percent of the agricultural area are under organic farming, and Austria is thus complying even today with the respective goals of the EU Strategies. About 80 percent of the Austrian farms are already participating in the Austrian Agri-environmental Programme ÖPUL. There must not be a "one fits all" model in this case. Moreover, the decline of biodiversity has to be looked at in a differentiated way. There is a wide variety of reasons, which must be looked for, not only in the field of agriculture, but also in other economic sectors and fields of politics. Therefore, measures of agricultural policy alone will not be sufficient to put a hold on biodiversity loss.

## Sound findings from research

A sustainable and economically successful agriculture needs modern methods and new findings also in the field of production and the use of high-quality feedstuffs. The know-how for this purpose is provided not least by the agencies of the Federal Ministry of Agriculture, Regions and Tourism and its outsourced institutions such as AGES (Austrian Agency for Health and Food Safety), which cooperate with partner organisations in Austria and abroad. The results are directly incorporated into practice. In the feed sector the focus is on sustainability, climate change, but also on efficiency and profitability. At the Agricultural Research and Education Centre Raumberg-Gunpenstein, for example, research is being made on the question how cattle can be made more climate compatible by reducing their methane emissions by means of certain feed additives. A feeding experiment has demonstrated that cattle emit 15 percent less methane if lemon grass is added to their feed. Now it is being investigated whether the domestic pulse sainfoin has a similar effect. The project "ClimGrassEco", as well from Raumberg-Gumpenstein, is investigating into the effects of drought and higher CO2 concentrations on grassland and is to provide findings for respective adaptation measures. The University of Natural Resources and Life Sciences Vienna has examined within the framework of the project "Improvement of forage efficiency in grassland-based, organic milk production" which kind of forage and which type of processing is best accepted by the cows, so that they need in total less concentrate.

## Innovative ideas for a future-proof feed production

With the Innovation Farm the competences in the field of digitalisation are bundled and prepared for agriculture in a comprehensible way. This "digital model farm" is destined to help to identify and to understand the new developments better and more rapidly in order to be able to generate as much benefit for the versatile agriculture in Austria as possible. The goal is a sustainable further development of agriculture by means of new technologies. The focus is on arable farming, grassland farming, and animal husbandry.

My goal as Minister of Agriculture is, involving the whole branch of industry, to continue to create framework conditions promoting innovation and progress in order to be able to guarantee in this way a sustainable and effective supply with feedstuffs.

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## Organizing Committee



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Head of the Organizing Committee of Feed 2021



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Technical assistance



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AGES event management



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## Scientific Committee

Our experts constitute a broad panel of experience in the field of feed science (alphabetic order):



Vincent Baeten  
Head of unit at Walloon Agricultural Research Centre, Belgium

Radmilo Čolović  
Technical expert for feed/aqua, Cargill CSST, Germany



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Lujia Han  
Full professor at the Department of the Agricultural Engineering, College of Engineering, China Agricultural University, Beijing, China



Christoph von Holst  
Scientific officer at the European Commission Joint Research Centre, operating manager of the European Union Reference Laboratory for feed additives, Belgium

Jacob de Jong  
Programme leader feed, Wageningen University and Research centre, The Netherlands



Rudolf Krska  
Full professor, Head of Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Science, Austria



Luciano Pinotti  
Full professor at the Department of Health, Animal Science and Food Safety, Fac. Veterinary science, University of Milan, Italy

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Ursula Vincent  
Scientific officer at the European Commission Joint Research Centre, Belgium

Jürgen Zentek  
Full professor, Head of institute of animal nutrition, Department of Veterinary Medicine at the Freie Universität Berlin, Germany



# CONFERENCE PROGRAM

All times are in Central European Time

Poster show and discussion are available online

## WEDNESDAY 23 JUNE 2021

### 09:00 Words of Welcome

Thomas Kickinger, Managing Director, AGES

### 09:15 Welcome speech

Ministry of Agriculture, Regions and Tourism, Austria

### 09:35 General keynote: Past and present challenges on the safety and efficacy assessment of feed additives

Frank Verdonck, EFSA

## SESSION 1 FEED SAFETY

**Chairperson:** Robin Ørnsrud (NIFES), Jörg Seifert (FEFANA)

### 10:05 Introduction

### 10:10 Keynote Speech: Update and outlook on regulatory developments and enforcement of feed safety at EU levels, with focus on undesirable substances in feed

Frans Verstraete, DG SANTE, European Commission

### 10:40 Carryover in feed production and transfer from feed to food of undesirable compounds in the animal food production chain - FAO expert meeting

Marc Berntssen, Institute of Marine Research, Norway

### 11:00 Break

### 11:30 Determination of cross-contamination levels for antimicrobial active substances regarding new EU-legislation

Robin S. Wegh, Wageningen Food Safety Research, The Netherlands

### 11:50 Determination of p-Phenetidine in feed: Analytical challenge for the safe use of ethoxyquin in animal nutrition

Ursula Vincent, European Commission, Joint Research Centre, Belgium

### 12:10 Metals and nitrogenous compounds in feed – compounds of analytical and regulatory interest

Heidi Amlund, National Food Institute Denmark, Denmark

### 12:30 Lunch break

### 13:15 Lab Tour Biomin

Gerd Schatzmayr, Biomin, Austria

**Chairperson:** Ursula Vincent (JRC),  
Rudolf Krska (BOKU-IFA-Tulln & FFOQSI)

### 13:45 Introduction

### 13:50 Advances in feed safety - whole genome sequencing of microbial contaminants

Werner Ruppitsch, AGES, Austria

### 14:10 Limitations of microbiological testing for *Salmonella* spp. and Enterobacteriaceae in field samples to assess feed safety

Erwin Witters, Kemin, The Netherlands

### 14:30 Rapid detection of residues from packaging remnants in former foodstuff products by multivariate analysis of RGB images

Matteo Ottoboni, University of Modena and Reggio Emilia, Italy

### 14:50 Detection of microplastic in feed ingredients

Leo van Raamsdonk, Wageningen Food Safety Research, The Netherlands

### 15:10 European standardization and how to be involved

Charlotte Mosies, NEN, The Netherlands

### 15:20 IAG Feed Microscopy Association and its work in establishing a standardized method of detection and quantification of packaging material in feed

Geneviève Frick, Agroscope, Switzerland

### 15:30 Break

## SESSION 2 FEED FRAUD AND FEED AUTHENTICITY

**Chairperson:** Vincent Baeten (CRA-W), Carsten Fauhl-Hassek (BfR)

### 16:00 Introduction

### 16:05 Keynote Speech: Enforcement of EU feed legislation with specific focus on truthfulness of feed labelling and feed fraud

Wolfgang Trunk, DG SANTE, European Commission

### 16:35 Vibrational spectroscopy and imaging coupled with chemometrics for the authenticity of protein feed: the example of antibiotic mycelia residues

Zengling Yang, China Agricultural University, China

### 16:55 DART mass spectrometry: A rapid tool for the identification of feed additives

Christoph von Holst, European Commission, Joint Research Centre, Belgium

### 17:15 Evaluation of the cross-reactivity between plasma peptides and mastitis milk for the development of multiple reaction monitoring mass spectrometry for bovine plasma powder detection

Marie-Caroline Lecrenier, Walloon Agricultural Research Center, Belgium

### 17:35 End of day 1



## THURSDAY 24 JUNE 2021

### SESSION 3 NATURAL TOXINS AND CLIMATE CHANGE

**Chairperson:** **Jacob de Jong (WFSR-WUR),  
Leo van Raamsdonk (WFSR-WUR)**

- 9:00 Introduction**
- 9:05 Keynote Speech: How to tackle natural toxins in view of globalisation and climate change**  
Rudolf Krska, University of Natural Resources and Life Sciences, Vienna and FFoQSI Competence Center for Feed & Food, Austria
- 9:35 Mycotoxin risks in stored Swedish grain**  
Erik Nordkvist, National Veterinary Institute Uppsala, Sweden
- 9:55 Classification of the maize assortment regarding the susceptibility to ear fusariosis – 10 year experience in AT**  
Elisabeth Viktoria Reiter, AGES, Austria
- 10:15 Mitigation of mycotoxin exposure with feed enzymes**  
Dieter Moll, Biomin, Austria
- 10:35 Application of the HRMS-QExactive for the development of a comprehensive mass spectral database for pyrrolizidine alkaloids**  
Ewelina Kowalczyk, National Veterinary Institute Pulawy, Poland
- 10:55 Break**

### SESSION 4 IMPACT OF FEED ON ANIMAL HEALTH AND WELFARE

**Chairperson:** **Luciano Pinotti (UNIMI), Christoph von Holst (JRC)**

- 11:15 Introduction**
- 11:20 Keynote Speech: Feed - Impact on physiology, health and welfare of farm animals**  
Jürgen Zentek, Freie Universität Berlin, Germany
- 11:50 Speciality Feed Ingredients' contribution to sustainable animal farming/pivotal role in the fight against Antimicrobial Resistance**  
Jörg Seifert, FEFANA
- 12:10 Studies on the mechanisms of action of phytogetic feed additives including Next-Generation Sequencing (NGS)**  
Klaus Teichmann, Biomin, Austria
- 12:30 Undesirable substances in interspersed substrates - impact on animal and consumer health**  
Felicitas Koch, BfR, Germany
- 12:50 Lunch break**

- 13:30 Lab Tour: The world of FFoQSI - Visit to the Austrian Competence Center for Feed & Food, Quality, Safety and Innovation**  
Rudolf Krska, Institute for Bioanalytics and Agro-Metabolomics, FFoQSI, Austria
- 14:00 European Feed Microbiology Organization - Promoter of microbiological feed quality**  
Manuela Zadravec, EFMO
- 14:10 Official Controls and Accredited Third Party Certification - Working together to secure the feed supply chain**  
Emmanouil Geneiatakis, FAMI-QS, Belgium

### SESSION 5 SUSTAINABILITY AND CIRCULAR ECONOMY

**Chairperson:** **Elisabeth Reiter (AGES), Anthony Verschoor (WFSR-WUR)**

- 14:20 Introduction**
- 14:25 Keynote Speech: FEFAC Charter 2030: A comprehensive view on sustainable feed production**  
Asbjørn Børsting, FEFAC
- 14:55 Effects of confectionary or bakery former food products as cereal substitute on growth performance in post-weaning piglets**  
Luciano Pinotti, University of Milan, Italy
- 15:15 Break**
- 15:35 Multi-omics analysis of Atlantic salmon liver tissue after exposure to pirimiphos-methyl**  
Josef D. Rasinger, Havforskninginstituttet, Norway
- 15:55 Extraction of functional nutritive derivatives from unicellular organisms by novel technologies for sustainable production of animal feed**  
Miladinovic, Dejan, Norwegian University of Life Science, Norway
- 16:15 Connecting agri-food supply chains through insect farming: upcycling underused materials into animal feed ingredients**  
Christophe Derrien, IPIFF
- 16:35 Poster prize**
- 16:45 Closing session**
- 17:00 End of conference**

## Keynote Speakers



Frank Verdonck



European Food Safety Authority



Frank Verdonck holds a Master's degree in biotechnology and a PhD in veterinary science, both from Ghent University (Belgium). He worked in academia and pharmaceutical industry before joining EFSA in 2012. He was first active in the team supporting the Panel on Animal Health and Welfare. From January 2019 onwards, he is the ad interim head of the FEED unit. He is responsible for the delivery of EFSA's opinion on the assessment of feed additives and is involved in the implementation of the Transparency Regulation at EFSA.



Frans Verstraete



Official, European Commission  
DG Health and Food Safety



Frans Verstraete graduated in 1985 as agricultural engineer at the University of Ghent (Belgium). After his studies he held positions at the University of Ghent and thereafter at the Belgian Ministry of Agriculture and he was for a period technical adviser of the Belgian Minister of Agriculture. He is working for the European Commission since 1997. In the European Commission he has had various functions and since 2000 he is working at the Directorate General Health and Food Safety. He is responsible for the elaboration, development and management of the EU-legislation on contaminants in feed and food.



Wolfgang Trunk



DG SANTE, Animal Nutrition,  
Veterinary Medicines



Wolfgang Trunk holds a Master's degree in agronomy and received his doctoral degree in farm management and evaluation of policy options of agricultural production based on market analyses from the University of Hohenheim, Germany. He worked as an administrator for economic aspects of German and EU milk production and dairy industry and also as administrator for economic relations to American countries at the Federal Ministry of Agriculture (Germany). After gaining experience as a national expert for the Common Agricultural Policy and rural development in the European Commission, he became attaché for the Common Agricultural Policy at the Permanent Representation of Germany to the EU.

Since 2005 he holds the position of the policy coordinator in the field of feed legislation (e.g. additives, dietary feed, feed materials, compound feed, medicated feed, at DG SANTE of the European Commission.



## Rudolf Krska



University of Natural Resources and  
Life Sciences, Vienna



University of Natural Resources  
and Life Sciences, Vienna

Rudolf Krska is full professor for (Bio-)Analytics and Organic Trace Analysis at the University of Natural Resources and Life Sciences, Vienna (BOKU). He is head of the Institute of Bioanalytics and Agro-Metabolomics the Department IFA-Tulln at BOKU. 2010 he worked for one year as A/Chief of Health Canada's Food Research Division. Prof. Rudolf Krska has received 11 scientific awards and is (co)author of more than 400 SCI publications (Scopus h-index: 65; >15.000 citations). Based on the world-wide impact of his papers he is ranked Nr. 4 in the area of food safety among more than 50.000 scientists ([expertscape.com](http://expertscape.com)).

Krska was coordinator of the European Commission funded project MyTool-Box for Integrated Mycotoxin Management in cooperation with China with a funding volume of more than 6 Mio Euro. Moreover, Krska is head of strategic research at the recently founded Austrian Competence Center for Feed and Food Quality, Safety and Innovation (FFOQSI) with a total funding volume of 28 Mio Euro. Since 2018, he is also affiliated with the Institute for Global Food Security at Queen's University, Belfast, UK where he holds a position as jointly appointed professor.



## Jürgen Zentek



Freie Universität Berlin, Germany



Jürgen Zentek received his degree as veterinarian in 1985 from the Veterinary school of Hannover. He received his doctoral degree in 1987, and a degree as specialist in animal nutrition and dietetics in 1993. He had an extraordinary professorship at the Hannover vet school from 1999-2001. After one year as research scientist in Bristol/UK, school of veterinary science, he was appointed on the chair of clinical nutrition at the Veterinary University of Vienna where he also became the head of the institute of nutrition. In 2005, he was appointed as professor at the Institute of Animal Nutrition, Freie Universität Berlin. The main research interests cover the role of nutrition for the intestinal microbiota and immunity of the gastrointestinal tract. Prevention of health disorders in domestic animals is one of the main topics of the institute with a specific focus on gastrointestinal diseases. Prof. Zentek had directed a collaborative research group on the mode of action of probiotics in pigs and has been involved in many European and national research projects on feed and food safety.



## Asbjørn Børsting

FEFAC President



Asbjørn Børsting was elected President of FEFAC for 2020–2023 at FEFAC's 65th General Assembly. Mr Børsting is a graduate of the University of Copenhagen (Agricultural Economics). He has served as CEO of The Danish Agricultural Council and as CEO in DLG. He is currently Director of DAKOFO, the Danish Grain and Feed Trade Association and Variety Denmark – a Danish Seed Association. He is also Chairman of the Danish Bio-Economy Panel.

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# Talks

## General Keynote: Past and present challenges on the safety and efficacy assessment of feed additives

Jaume Galobart, Montserrat Anguita Freixa, Paola Manini, Maria Vittoria Vettori and Frank Verdonck (presenter)

Thirty years ago, outbreaks of Bovine Spongiform Encephalopathy (BSE) and presence of dioxins in feed triggered the implementation of specific risk assessment methodologies to ensure and strengthen human and animal protection in Europe. The European Food Safety Agency (EFSA) was established and the FEEDAP Panel has adopted more than 1100 scientific opinions on the safety and efficacy of feed additives. The risk assessment framework has been recently modified based on society demands (Transparency Regulation) and will likely be further adapted to ensure sustainability (Green Deal). Technological developments influence the characterisation of microorganisms while new theoretical concepts created a basis to assess complex products such as botanicals. A challenge ahead is how to assess the efficacy of new functional groups of feed additives that influence other end points than strict animal production parameters. In addition, the application of novel feed is approaching and the reuse of food no longer intended for human consumption will need to be evaluated.

## Session 1 Feed Safety

### Keynote: Update and outlook on regulatory developments and enforcement of feed safety at EU levels, with focus on undesirable substances in feed

Frans Verstraete

The EU legislation on undesirable substances in feed has ensured already for nearly 50 years a high level of animal and public health and the environment. However, a continuous update is needed to take account of the development of scientific and technical knowledge and to address new challenges e.g. as the consequence of climate change. The presentation will provide an overview of recent regulatory developments, ongoing discussion and provide an outlook to the future. A wide range of topics are currently under discussion: Fusarium mycotoxins (deoxynivalenol, T-2 and HT-2 toxin, zearalenone, fumonisins), ochratoxin A, ergot alkaloids, dioxins and PCBs, PFAS, tropane alkaloids, pyrrolizidine alkaloids, THC, nitrites/nitrates, ...). Enforcement of legislation is as important as the setting of regulatory limits to ensure the safety of feed. The current legislation on sampling and methods of analysis is currently under review, also providing for an effective enforcement of feed traded on-line. The presentation shall provide more details and background information on these aspects clarifying the why and how of the regulatory provisions.

## Carryover in feed production and transfer from feed to food of undesirable compounds in the animal food production chain- FAO expert meeting

Marc H.G. Berntssen

In 2019 WHO-FAO published a report; "Carryover in feed and transfer from feed to food of unavoidable and unintended residues of approved veterinary drugs". Carry over refers to the unintended cross contamination of veterinary residue during animal feed production, transport, or use at farm sites. The use of alternative feed ingredients (e.g. processed animal proteins PAP) can be another unintended source for pharmaceuticals residues in animal feeds. Transfer refers to the following transfer from cross contaminated feed to the edible parts of food producing animals. It was concluded that the unintended carryover of veterinary drug is unlikely to give food concentrations higher than when the veterinary drug is used at therapeutic dose for target animals. However, human health risks cannot be ruled out and specific risk assessments may be required to determine if the level of carryover results in food residues can exceed acceptable daily intakes for consumers. Antimicrobial residues at carryover concentrations in animal feed may require additional consideration for antimicrobial resistance.

Carryover of veterinary drugs during animal feed production is in some cases unavoidable even if the Codex Code of Practice on Good Animal Feeding, GMP, and HACCP principles are followed. The WHO-FAO report stated that an acceptable amount of drug could be established based on the residue tolerances (i.e., MRLs) in the subsequent food products from exposed target animals. However, for many veterinary drugs no MRLs have been established for non-target species/products. It was suggested to establish action levels for none target animals based on the following considerations: A) Drug carryover during feed production or pharmaceutical residues present in feed ingredients. B) Identify action level in feed for non-target species. C) Determine transfer from feed to food. D) Determine action level for food products from non-target species. In this presentation, an example is given of the unintended introduction of pharmaceuticals in seafood production when processed animal proteins (PAP) are being used in aquafeeds. Proposed action levels for decision making are given based on wide scope analytical screening results and transfer to the edible part of the farmed fish assessed by physiological based toxico-kinetic (PBTK) transfer models.

## Determination of cross contamination levels for antimicrobial active substances regarding new EU legislation

Robin S. Wegh, Erik de Lange, Tina Zuidema, Jacob de Jong

Different compound feeds have to be manufactured in the same production line. As a consequence, traces of the first produced feed may remain in the production line and get mixed with the next feed batches. This "cross-contamination" is unavoidable, and so non-medicated feed can be contaminated with veterinary drugs like antibiotics added to the previous batch of medicated feed.

Maximum levels of cross-contamination for antimicrobial active substances in non-target feed need to be established according to new European Union legislation for medicated feed [1]. With these new rules, methods of analysis for antimicrobial active substances in feed, as listed in Annex II of Reg. (EU) 2019/4, are needed at relevant levels. At WFSR, multiple methods are operational for the analysis of these substances in feed. An overview of these methods will be presented.

Next to presenting available methods for the analysis of the active substances mentioned above, research on cross-contamination in feed production will be presented [2]. To monitor the cross-contamination of antibiotics in the Netherlands, 21 feed mills were visited and 140 samples of flushing feeds were collected and analyzed for residues of antibiotics. Results show that 87% of all samples contain concentrations of antibiotics in the range of 0.1-154 mg/kg.

[1] REGULATION (EU) 2019/4 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL, of 11 December 2018. on the manufacture, placing on the market and use of medicated feed, amending Regulation (EC) No 1831/2003 of the European Parliament and of the Council and repealing Council Directive 90/167/EEC

[2] Stolker AAM, Manti V, Zuidema T, van Egmond H, Deckers ER, Herbes R, Hooglugt J, Olde Heuvel E, de Jong J. 2013. Carry-over of veterinary drugs from medicated to nonmedicated feeds in commercial feed manufacturing plants. *Food Addit Contam A.* 30:1100–1107.

## Determination of p-Phenetidine in feed: Analytical challenge for the safe use of ethoxyquin in animal nutrition

Ursula Vincent, Federica Serano, Christoph von Holst

Ethoxyquin has been being used for a long time as antioxidant mainly for fishmeal, but also for feedingstuffs and feed additive formulations containing vitamins and carotenoids. Ethoxyquin contains as impurity p-phenetidine, which is a mutagen and based on this fact the European Food Safety Authority (EFSA) could not conclude on the safety of this product. Furthermore, EFSA did not propose any maximum limits of the impurity in ethoxyquin. In between DG SANTE issued Regulation 2017/962 suspending the authorisation of ethoxyquin as a feed additive for all animal species and categories. In addition the industry applying for an EU authorisation for placing this additive on the market are requested to submit additional data, including the development of fit for purpose analytical methods for p-phenetidine. The latter aspect refers to the determination of the impurity p-phenetidine in the matrices feed additives, premixtures, feed and fishmeal. The target levels of p-phenetidine in the ethoxyquin that the industry has to achieve is 2 mg/kg, which translates into maximum legal limit of p-phenetidine in feed of 125 ng/kg, when authorising ethoxyquin with a maximum content of 50 mg/kg in feed. When the risk assessment and the analytical developments will be completed limits for p-phenetidine in the various matrices will be set under the "undesirable substances Regulation".

In this presentation, the achievements on a method of analysis developed and single-laboratory validated for the determination of p-phenetidine in fish meal are discussed as well as the challenged encountered. Potential follow-up pending on policy directions are also displayed.

### Author information

Team leader control activities of the EURL for Feed Additives at the European Commission Joint Research Centre. More than 20 years experience in analytical method development and validation.

## Metals and nitrogenous compounds in feed – compounds of analytical and regulatory interest

Heidi Amlund, Rasmus la Cour, Lucas Givelet, Kit Granby and Jens J. Sloth

The National Food Institute hosts the European Union reference laboratory for metals and nitrogenous compounds in feed and food (EURL-MN). The EURL-MN competences includes all metals, other elements, and nitrogenous compounds in feed and food. European Union reference laboratories (EURLs) are expert laboratories designated by the European Union (EU). The EURLs contribute to the improvement and harmonisation of analytical methods used by official control laboratories. In the EU, maximum levels (MLs) are set for a range of undesirable substances, including total arsenic and nitrite, in feed and feed ingredients (EU Directive 2002/32 and amendments).

There is currently a focus on undesirable substances not included in the legislation, and on some of the compounds already included. Compounds of interest are nickel, inorganic arsenic, nitrite and nitrate. Following a call for data and subsequently new occurrence data for nickel in feed and food, the European Food Safety Authority (EFSA) is updating their former exposure assessments. EFSA is also preparing an opinion on nitrate and nitrite in feed. The coming scientific opinions of EFSA may require regulatory follow up by the EU. An EU Commission recommendation of the monitoring is inorganic arsenic in feed is under adoption. Good monitoring requires good analytical capabilities of the laboratories.

Compounds of regulatory interest will be discussed with regards to their relevance for feed safety, methodology, and the analytical capability of the laboratories in the EURL-MN network. The review of the methodology will focus on the development of analytical methods for the determination of inorganic arsenic, nitrite and nitrate in feed.

### Author information

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 European Reference laboratory for metals and nitrogenous compounds

## Advances in feed safety - whole genome sequencing of microbial contaminants

Werner Ruppitsch<sup>1</sup>, Aleksandra Martinovic<sup>2</sup>

Microbial contamination of the feed-food chain is a major global health threat. Surveillance is an indispensable requirement for early detection of microbial threats, which allows the timely implementation of appropriate measures to terminate outbreaks, prevent further transmission and morbidity. In our today's global world, where pathogens easily and rapidly cross national borders, pathogen monitoring requires efficient local, national and international surveillance systems.

The rapid progress in sequencing technology from Sanger sequencing to whole genome sequencing (WGS) or next generation sequencing (NGS) has revolutionized the field of microbiology. The superiority of WGS based strain characterization has led to the replacement of former gold standard typing tools like for example fluorescent amplified fragment length polymorphism (FAFLP), pulsed-field gel electrophoresis (PFGE), multiple-locus variable number tandem repeat analysis (MLVA) and serotyping. WGS-based typing based on either single nucleotide variants (SNVs), on gene-by-gene allelic profiling using core genome multilocus sequence typing (cgMLST) or whole genome multilocus sequence typing (wgMLST) is currently the most powerful tool to characterize microorganisms. The general benefits of diverse WGS based strain typing approaches compared to traditional methods are robustness, superior discriminatory power, possibility to infer the geographic origin, and to obtain evolutionary information, data about virulence and antimicrobial resistance of outbreak isolates. WGS allows today high-throughput and inexpensive analysis of entire bacterial genomes. In addition, the high data quality, the reproducibility and accuracy of WGS technology has been demonstrated. For backward compatibility to datasets obtained with traditional methods information on serotype, classical multilocus sequence type (MLST) or MLVA data can be extracted from WGS data.

WGS allowed the creation of global databases based on standardized nomenclatures, like the current MLST databases. The benefit of global databases is the global exchange of data as a prerequisite for improving outbreak investigation, strain tracking, identification of the source of infection.

With further technological advancement, genomic approaches may provide future solutions for a complete pathogen detection and pathogen characterization directly from specimens.

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## Limitations of microbiological testing for *Salmonella* spp and Enterobacteriaceae in field samples to assess feed safety

Erwin Witters, Carmen Coetzee, Ellen Nobels, Karen Bierinckx, Lieselot Wouters, Sayu Ravichandran, Roland Accoe and Luis Conchello

**Objective:** Today, correct recommendations on effective doses of feed safety additives are a challenge in the feed industry. It is key to understand the limitations of the scientific and systematic approaches on which feed safety declaration can be based. Here, data obtained from natural contaminated field samples are combined with knowledge and insights from artificial contaminated feed matrices to create a lab service that can be used to assist feed safety management.

**Methods:** Classic Total Enterobacteriaceae Count, Total Coliform, *E. coli*, *Salmonella* positive-negative detection, Most Probable Number assays and Total Mould Count are carried out to assess natural contamination on a variety of feedstuff samples coming from the field. Bacterial strains are isolated from field samples. Bioscreen tests are used to assess antimicrobial sensitivity. Optimized inoculation spray techniques are used to create artificial contaminated feedstuff samples. Advanced application techniques synonymous to field applications are used to treat contaminated samples with organic acid based antimicrobials. Feedstuff parameters tested include: various feedstuff matrices, feedstuff temperature, microbial load, acid-binding capacity (pH), moisture and water activity. Experimental variables tested include: microbial distribution (random, regular, irregular and clustered), sampling strategy, temperature, antimicrobial dose and microbial kill off dynamics.

**Results and Discussion:** A period 10 years of data collection resulted in a database containing 40 000 microbial analyses entries and 5 000 physicochemical test entries coming from compound feed and raw materials. Statistical analysis is used to reveal trends, associations and correlations between microbial entities, microbial prevalence, physicochemical parameters and type of feedstuff with the aim to reveal enhanced risk factors and to assign surrogate markers that are easy to monitor and implement in an industrial setting. Recalcitrant matrices (eg high protein soybean meal, rapeseed meal), irregular distribution (eg contamination clusters and hot spots), temperature variations, *Salmonella* status or metabolic activity, water activity and moisture variations challenge the efficacy of the applied antimicrobial to a significant extent. Advancements include modelling the risk assessment affecting feed safety to then ensure practical application techniques and technologies, dosages and the relevant antimicrobial is combined to effect the positive outcome for safe feed.

### Author information

Erwin Witters is an R&D professional heading the Customer Laboratory Services of Kemin Animal Nutrition and Health EMENA.



## Rapid detection of residues from packaging remnants in former foodstuff products by multivariate analysis of RGB images

Alessandro Ulrici, Rosalba Calvini, Alice Luciano, Matteo Ottoboni, Marco Tretola, Luciano Pinotti

In this work, we present an investigation of the potentialities of applying multivariate image analysis (MIA) techniques to RGB images in order to detect packaging remnants in former foodstuff products (FFPs). In particular, multivariate image analysis was conducted considering two different approaches, i.e. pixel-level analysis of single images and image-level analysis of image datasets. Pixel level MIA was applied both to the raw RGB images and to the "augmented" RGB images, which were obtained by including additional colour-related parameters. The use of augmented RGB images allowed to better highlight differences between particles of packaging remnants and the ex-food matrix, in particular when their colour was similar. Image-level approach allowed to simultaneously compare all the images of the dataset, highlighting both groups of images with similar colour-related features and outlier images, e.g. those containing packaging particles. The results demonstrate the feasibility of using pixel-level and image-level MIA as a rapid automated method for detecting particles of packaging materials in FFPs, in particular when the colour of the undesirable packaging remnants can be differentiated from the colour of the ex-food matrix.

### Author information

Alessandro Ulrici is associate professor of Analytical Chemistry at the Department of Life Sciences, University of Modena and Reggio Emilia, Italy.

## Detection of microplastic in feed ingredients

L. van Raamsdonk, N. Dam, B. Hedemann, R. Peters

The presence of microplastic in the feed and food production chain attracts increasingly attention. Some data are available on concentrations in seafood (fish, bivalves and shrimps), water, sugar, salt and honey, but are lacking for a range of other feed and food materials. There are three dimensions which need attention with respect to monitoring: health effects, occurrence in relevant materials, and the development and quality assurance of detection methods. Well-developed detection methods are only available for a limited set of matrices, such as water, bivalves and salt. In order to develop data for contamination levels in feed and food products, dedicated analytical methods are needed.

WFSR has invested in the development of analytical methods for detection of microplastic in food and feed. Detection was aimed at the size range 1-300 µm for their toxicological relevance. This choice implied that a microscopic procedure was necessary to include, and results can only be quantified in terms of counts instead of weights. A strategy was needed for reproducible quantification of numbers of MP particles. A protocol was developed including steps for sample treatment (removal of matrix material), staining of particles by means of Nile Red (fluorescent dye), and standardised detection of the filtered sample material by using three different UV fluorescence filters. First results show that method development for and examination of a variety of MP particles in sturdy matrices is complicated.

Results will be placed in the wider framework and requirements for method development, quality assurance and quality control.

Raamsdonk, L.W.D. van, M. van der Zande, A.A. Koelmans, L.A.P. Hoogenboom, R.J.B. Peters, M.J. Groot, A.A.C.M. Peijnenburg, Y.J.A. Weesepeel, 2020. Current insights into monitoring, bioaccumulation, and potential health effects of microplastics present in the food chain. *Foods* 9: 72-99; doi:10.3390/foods9010072

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<https://www.wur.nl/en/Research-Results/Research-Institutes/food-safety-research.htm>

## European standardization and how to be involved

Charlotte Mosies

The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. The International Standards Organization (ISO) is the global equivalent. CEN's 34 National Members work together to develop European Standards and other deliverables in a large number of sectors to help build the European internal market in goods and services, removing barriers to trade and strengthening Europe's position in the global economy. More than 50.000 technical experts from industry, associations, public administrations, academia, and societal organizations are involved in the CEN network that reaches over 600 million people. We work in a decentralized way. Our members – the National Standardization Bodies (NSBs) of the EU and EFTA countries – operate the technical groups that draw up the standards; the CEN-CENELEC Management Centre (CCMC) in Brussels manages and coordinates this system. European Standards (ENs) are based on a consensus, which reflects the economic and social interests of 34 CEN Member countries channelled through their National Standardization Organizations. The development of a European Standard (EN) is governed by the principles of - consensus, openness, national commitment and technical coherence. All stakeholders can take the initiative to develop a European Standard. The work is done by experts in technical bodies, such as CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis" and its WGs. It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements.

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## IAG Feed Microscopy Association and its work in establishing a standardized method of detection and quantification of packaging material in feed

Geneviève Frick, Leo van Raamsdonk, Roland Weiss, Jeroen Vancutsem, Renate Krull-Wöhrmann, Ragna Schadewaldt, Tina Eggers

Microscopy is an important legal tool for enforcement of regulations, with the mere notification that some of those regulations can be enforced only by microscopic inspection. In addition, the results of microscopic investigations are in a range of situations complementary to analytical chemical research. Multidisciplinary research is necessary in most cases in order to clarify and understand obtained results, and to trace causes and origins for pro-active and risk-based monitoring. In this respect, microscopy-based observation is pivotal.

In contrast to the instrumental analysis of analytic chemistry, the visual identification of a large diverse range of materials relies heavily on the knowledge of biology and adjacent disciplines. The analyst can gain routine in a day-by-day process of investigating sample material, which is a long and intensive process. Sources for expertise can be found among handbooks, training by experienced scientists, collections of reference materials and software-assisted expert systems.

Specific examples of microscopic, or in broader terms, visual research include detection of animal proteins (Regulation (EC) 999/2001), of botanic impurities (Directive 2002/32/EC), label control (Regulation (EC) 1169/2011), customs (Regulation (EU) 1308/2013) and of prohibited ingredients such as packaging materials (Regulation (EC) 767/2009). In addition, detection of micro-plastics is getting attention.

Packaging materials can harm animals or disturb their feed intake, pollute the environment and can be considered an undesired impurity in feeds. These materials do not consist of a definite molecule, group of molecule, living species or definite bodies. They can be plastic foil, hard plastic, metal pieces, paper, wood, or some combination of materials. This is a typical topic for microscopy.

In this presentation, the importance of the IAG Feed Microscopy Association is highlighted by the example of the work done on detection of packaging materials:

- "alert" since 2012 by IAG members (increase in food-recycling)
- presentation of monitoring results at annual meetings
- method comparison and development
- first collaborative study in 2016
- workshop in the frame of the annual meeting 2017
- distribution of training samples in 2018
- proficiency testing with 32 participants in 2019

### Author information

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## Session 2 Feed Fraud and Feed Authenticity

### **Keynote:** Enforcement of EU feed legislation with specific focus on truthfulness of feed labelling and feed fraud

Wolfgang Trunk

Regulation (EC) 767/2009 established the EU rules for feed marketing. A specific chapter is dedicated to the principles of labelling. Whereas the enforcement of feed safety is well established throughout the EU, the truthfulness and authenticity of the labelling is increasingly at risk, and the customers are misled, in particular in the area of pet food. The intention of such practice is often to have an economic profit. The Commission has therefore expanded the established alert system for food fraud also to incidents with feed. The presentation explains the legal framework and the respective EU actions to fight such practices.

### Vibrational spectroscopy and imaging coupled with chemometrics for the authenticity of protein feed: the example of antibiotic mycelia residues

Zengling Yang, Shouxue Li, Erwei Liu, Zhuolin Shi, Xian Liu, Lujia Han\*

Antibiotic mycelia residues (AMRs) are the by-products of microbial fermentation for the production of antibiotics. AMRs are rich in nutrients, such as high crude protein content. However, AMRs contain trace amounts of antibiotics and have not been adequately evaluated for safety, so there is a risk of inducing resistant bacteria. If AMRs are illegally used as feed materials, which will cause bacteria in the animal to become resistant because of prolonged contact with antibiotics. For these reasons, all types of AMRs have been forbidden using as feed materials since 2002 in China and listed in the national hazardous waste list in 2008. At present, the detection of AMRs in feed is mostly aimed at detecting the remaining antibiotics using HPLC, HPLC-MS/MS, GC-MS, and HPLC-TOF/MS. These methods can sensitively detect the content of antibiotic in feed. However, the type of antibiotics in the feeds needs to be known in advance, and the methods are time-consuming and expensive. Vibrational spectroscopy, commonly associated with IR (including NIR and MIR) absorption and Raman scattering, has provided a powerful approach for investigating contaminants, foreign bodies and fraud in food and feed, especially with new developments in spectral imaging and chemometric. This research will take the soybean meal, cottonseed meal, and DDGS as examples for protein feed, and take the oxytetracycline residue (OR), streptomycin sulfate residue (SR) and colistin sulfate residue (CR) as examples for AMRs to investigate the feasibility to identify the AMRs in protein feed by combining vibrational spectroscopy and imaging techniques in a complementary manner. NIR and MIR microscopic image and surface enhanced Raman scattering (SERS) was investigated respectively. Different chemometrics methods, including Characteristic peaks identify, local anomaly detection, one-class partial least squares analysis (OCPLS) et al, were selected to mine spectral and image data. The results indicated: (1) Vibrational spectroscopy and imaging coupled with chemometrics could identify the AMRs or antibiotics in protein feed successfully. (2) Combining vibrational spectroscopy and imaging techniques allowed for an improved diagnostic reliability due to the complementary nature of retrieved information. (3) Applying chemometric algorithms to vibrational spectra dramatically improved the discriminative power.

#### **Author information**

Yang Zengling, Professor of China Agricultural University, has more than 10 years' experience on detection of feed using molecular vibration spectrum and chemical imaging and about 80 papers published.

## DART mass spectrometry: A rapid tool for the identification of feed additives

Christoph von Holst, Ursula Vincent, Mostafa Chedin, Diego Alonso Albarracin, Georgios Kaklamanos

Feed additives are widely used in animal nutrition, but require pre-market authorisation within the European Union. The authorisation procedure foresees a risk assessment for each product, conducted by the European Food Safety Authority and an evaluation of the analytical methods for the determination of the feed additives in various matrices by the European Union Reference Laboratory for Feed Additives. Based on corresponding evaluation reports, the European Commission decides on the approval or denial of the request for authorisation. Regulations authorising the product specify the conditions of use including the target levels of the active substance in the product and in compound feed. Important zotechnical feed additives are preparations of coccidiostats that are key substances utilised against the spread of coccidiosis, which is an animal disease, mainly observed in chickens and cattle. These feed additives are preparations containing various excipients such as carriers and one or two specific coccidiostats as active substances. Moreover, these feed additives are separately authorised, specifying in the Regulations their composition in terms of the content of the active substance and the excipients, thus requiring the availability of appropriate methods to check samples from the market against these provisions. While there are standard methods to determine the content of the active substance in the products, corresponding methods still need to be developed to establish whether specific preparations, considering also the excipients, are really authorised under specific regulations.

In this presentation the suitability of "Direct analysis of real time" (DART) mass spectrometry for the classification of feed additives containing coccidiostats is discussed. The analytical procedure involves the rapid extraction of the samples with organic solvents and subsequent measurement of the extracts with DART mass spectrometry, without employing any separation by liquid chromatography. The experimental design of the study includes the variation of the extraction solvents (polar versus apolar) and the operation of the mass spectrometer (positive versus negative). The obtained mass spectra are subsequently subjected to various multivariate statistics techniques.. The purpose of the talk is to present the results of the study and to elaborate on the potential of DART mass spectrometry in this field.

### Author information

Christoph von Holst is an analytical chemist with 22 years experience in feed analysis

## Evaluation of the cross-reactivity between plasma peptides and mastitis milk for the development of multiple reaction monitoring mass spectrometry for bovine plasma powder detection

M.C. Lecrenier<sup>1</sup>, J. Henrottin<sup>2</sup>, L. Plasman<sup>1</sup>, A. Cordonnier, N. Gillard<sup>2</sup> and V. Baeten<sup>1</sup>

In the context of the reintroduction of animal by-products in animal feed, current analytical schemes for the feed control have to be reviewed. One of the most promising methods is mass spectrometry-based proteomics.

The objective of this work was the selection of specific peptides for the detection of bovine plasma powder in feed. The selection of targeted proteins was done based on previous research (Lecrenier, 2016) and literature review (Steinhilber, 2018-2019). Candidate peptides were identified in silico by using the open source Skyline software under strict conditions including peptide length and amino acid modifications. Peptides specificity was evaluated against the entire NCBI nr database. Sensitivity was then evaluated on three types of compound feed (pig, poultry and aqua feed) adulterated with 0.1 to 2.5 % (w/w) of bovine plasma powder.

As several publications on mastitis milk have shown that plasmatic peptides could be present in mastitis milk, cross-reactivity was verified by analyzing feed spiked with fresh or lyophilized milk with low or high somatic cell counts (SSCs). Indeed, while the bovine plasma powder is not authorized in animal feed, milk powder is commonly used and mastitis milk could give false positive results in the context of the detection of feed materials unauthorized animals.

Proteins were extracted in a buffer containing 200 mM TRIS-HCl pH 9.2, 2 M urea followed by trypsin digestion and purification with tC18 SPE (Waters). Analyses were performed by liquid chromatography (Acquity UHPLC system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQ-XS, Waters).

The selected peptides were able to detect bovine plasma powder at 0.1 % (w/w) in all matrices. However, cross-reactivity was observed for several peptides in presence of milk and the signal intensity appeared to be independent of the presence of mastitis.

### Author information

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## Session 3 Natural Toxins and Climate Change

### **Keynote:** How to tackle natural toxins in view of globalisation and climate change

Rudolf Krska

The Food and Agriculture Organization (FAO) estimated the global food crop contamination with mycotoxins to be 25%. In order to assess the rationale for this figure which dates back to prior 1985, the relevant literature was reviewed and data of around 500 000 analyses from the European Food Safety Authority and large global survey for aflatoxins, fumonisins, deoxynivalenol, T-2 and HT-2 toxins, zearalenone and ochratoxin A in cereals and nuts were examined by M. Eskola et al. 2019. Using different thresholds, i.e., limit of detection, the lower and upper regulatory limits of European Union (EU) legislation and Codex Alimentarius standards, the mycotoxin occurrence was estimated. Impact of different aspects on uncertainty of the occurrence estimates presented in literature and related to our results are critically discussed. Current mycotoxin occurrence above the EU and Codex limits appears to confirm the FAO 25% estimate, while this figure greatly underestimates the occurrence above the detectable levels (up to 60-80%). The high occurrence is likely explained by a combination of the improved sensitivity of analytical methods and impact of climate change. It is of immense importance that the detectable levels are not overlooked as through diets, humans are exposed to mycotoxin mixtures which can induce combined adverse health effects. However, it is equally important to develop and apply integrated strategies to mitigate the mycotoxin issue along food and feed chains. This paper will thus also summarize the outcomes of the EU funded project MyToolBox ([www.mytoolbox.eu](http://www.mytoolbox.eu)) and suggest novel ways to reduce and control mycotoxins.

Eskola, M; Kos, G; Elliott, CT; Hajšlová, J; Mayar, S; Krska, R; (2019): Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited "FAO estimate" of 25. *Crit Rev Food Sci Nutr.* 2019; 1-17

### Mycotoxin risks in stored Swedish grain

Erik Nordkvist & Annica Tevell Åberg

Mycotoxins are a large (several hundreds) group of secondary metabolites produced of moulds (filamentous fungi). Several mycotoxins are common contaminants in feed and food and some, like aflatoxins are among the most toxic substances in nature while others have less well described effects.

We have studied handling and storage of cereal grain in Swedish primary production with focus on mycotoxins (primarily aflatoxins, AF, and ochratoxin A, OTA) via interviews, sampling and analysis of mycotoxins. Findings from these studies will be presented along with a few incident reports regarding traces aflatoxin via infected domestic cereal grain.

Aflatoxins are strictly regulated in food and AFB1 is the only mycotoxin with a maximum permitted level in animal feed. A metabolite of AFB1 is AFM1 which will contaminate milk if dairy cows are exposed to AFB1. Ochratoxin A is nephrotoxic and possibly carcinogen. The toxin is potential hazard in pig production and, since it is stored in edible tissue such as kidneys and muscle OTA in animal feed also may be a food hazard.

Temperature optimum for production of AF by *Aspergillus flavus* and *A. parasiticus* is 25-30 °C which makes the risk for contamination lower in cool and temperate climate such as in Sweden. Historically incidents with AFB1 contaminated cereals occurred with formic acid treated grain in the 1980s. Since then treatment propionic acid has been the choice of method for storage of moist cereal grain. Recent incidents have, however, shown that this treatment needs special attention and strict routines in order to prevent formation of aflatoxins. OTA on the contrary, predominantly produced by *Penicillium roqueforti*, may be produced at temperatures below 10 °C and is thus to be considered a more likely to occur contaminant under Swedish conditions. There have been incidents with OTA from time to time and then predominantly with cool-air dried grain, a method more sensitive to weather than hot-air drying. Today drying of cereals with ambient or just over ambient temperatures again are becoming more popular using so-called silo dryers, a design originating from different weather condition than Swedish.

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## Classification of the maize assortment regarding the susceptibility to ear fusariosis – 10 year experience in Austria

Elisabeth Viktoria Reiter, Klemens Mechtler, Hans Felder, Oliver Alber, Irmengard Strnad

The fusarium mycotoxins deoxynivalenol (DON), zearalenon (ZEA) and fumonisins (FUM) are known as the most dominant mycotoxins in Austria. Although the visual susceptibility for ear rot was already part of the registration procedure of maize varieties, the mycotoxin contents were not taken into account until 2013. Within a three-year-project a methodology was developed that considers also the mycotoxin contents. This method is now part of the annual registration procedure.

In total, about 1000 maize samples (from existing varieties and candidates for registration) are taken annually at over 30 different locations within the three most important climatic zones in Austria. The samples are analyzed for their DON, ZEA and FUM content by ELISA and the affected cob surface is checked visually. The obtained results by visual inspection are set into relation to the calculated local median values of the mycotoxin levels. The so calculated relative values are transferred into a 9-stage scale. The grades obtained at different locations are statistically combined over different environments to a variety specific grade for each criterion. The present methodology indicates that more robust varieties are identified resulting in lower mycotoxin contents especially in years with critical weather conditions. Farmers benefit of the obtained results because they are now able to select the most suitable maize variety based on their location in Austria and the possible mycotoxin susceptibility. In addition an early warning system has been developed, where samples are taken from fields prior to the harvest to give an early overview of the annual mycotoxin contamination.

### Author information

Elisabeth Reiter is the Head of Department for Feed Analysis and Quality Testing. She is an expert feed safety and quality of feed and feed derived products.

## Mitigation of mycotoxin exposure with feed enzymes

Dieter Moll, Markus Aleschko, Sebastian Fruhauf, Michaela Thamhesl, Heidi Schwartz-Zimmermann, Elsa Kunz-Vekiru, Gerd Schatzmayr

According to an annual, global mycotoxin survey conducted by BIOMIN, most animal feed is contaminated with one or more mycotoxins. Concentrations that are known to affect health and productivity of farmed animals are not unusual. Therefore, at the BIOMIN Research Center, we have been working on feed additive technology for mitigation of mycotoxin exposure. Common anti-mycotoxin feed additives contain binding agents for mycotoxin adsorption, and binding technology has been verified to work for mitigation of aflatoxin exposure. However, adsorbing agents were less effective for Fusarium mycotoxins including fumonisins, deoxynivalenol and zearalenone. Therefore, we have been researching and developing the use of enzymes for biotransformation and detoxification of such mycotoxins. Feed enzyme technology for mycotoxin mitigation has arrived for commercial application with the EU registration of the fumonisin esterase FUMzyme®. The enzyme is active in the gastrointestinal tract of animals and catalyses hydrolytic cleavage of the two tricarballic acid side chains from the core chain of carcinogenic fumonisins. Without these side chains, fumonisins no longer have the binding affinity to block their molecular target, ceramide synthase. Inhibition of ceramide synthase in animals causes concentrations of the precursors sphinganine and sphingosine to go up, and these sphingolipids can be analysed in blood samples from animals exposed to fumonisins to measure detoxification with FUMzyme®. FUMzyme® is derived from a soil bacterium with a fumonisin degradation gene cluster, and it is now produced by recombinant gene expression with high yield at large scale. The enzyme has also been engineered for thermostability so that it can tolerate the feed pelleting process. Recently, an instantly soluble version of FUMzyme® has been developed for liquid spray application to feed. Research and development on enzymes for detoxification of other mycotoxins is at various project stages. Most advanced is a zearalenone lactonase with the designated trade name ZENzyme®. Enzymes for mycotoxin degradation have the potential to further support efficient use of agricultural resources, and health and productivity of farmed animals.

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## Application of the HRMS-QExactive for the development of a comprehensive mass spectral database for pyrrolizidine alkaloids

Ewelina Kowalczyk<sup>1</sup>, Patrick Mulder<sup>2</sup>, Krzysztof Kwiatek<sup>1</sup>

Pyrrolizidine alkaloids (PAs) are a large group of natural toxins that are widely distributed worldwide.

It has been estimated that more than 6000 plant species contain these compounds. Chronic ingestion of PAs through contaminated feed like hay, straw or silage is the main source of livestock poisoning. Additionally, it was proved that PAs and their metabolites are not only toxic but also cancerogenic and teratogenic to many animals.

For the determination of PAs in feeds, mostly LC-MS/MS methods are used. However, this technique poses some limitations, as only PAs included in the method can be determined. Even though the number of the available analytical standards systematically increases, still only small percent of PAs is covered with the appropriate standards. Analysing the restricted number of PAs with targeted LC-MS/MS methods can lead to underestimation of the PAs content.

Liquid chromatography full scan high resolution mass spectrometry (LC-HRMS) does not have this limitation as it offers non-targeted analysis. HRMS poses a great potential for fast screening of samples on the presence of a wide range of PAs. However, to receive reliable identification of the compounds the database containing comprehensive information is required. For this reason, the aim of the study was the development of a broad database on pyrrolizidine alkaloids containing detailed information such as name, accurate mass, elementary composition, retention time and accurate masses of the characteristic fragments. The data has been collected using the LC-HRMS non-target method applying full scan and variable data independent acquisition modes. Over 60 PAs standards and extracts from around 200 plant species were available for the analysis.

Analysis of standards allowed to determine the characteristic fragmentation pattern of monoesters, diesters, macrocyclic diesters, corresponding N-oxides, otonecine and platynecine type PAs. Identification of the characteristic fragments and determination of their accurate masses allowed the development of the screening method enabling easy and effective detection of compounds potentially belonging to the class of PAs in analysed plant extracts. As a result, a broad number of PAs has been tentatively identified, and recorded in the developed database with all required detailed spectral information.

### Author information

Dr Ewelina Kowalczyk is an assistant professor in the National Veterinary Institute in Puławy, Poland.

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## Session 4 Impact of Feed on Animal Health and Welfare

### Keynote: Feed - impact on physiology, health and welfare of farm animals

Jürgen Zentek

Good nutrition must meet the nutritional needs of high performing farm animals; while on the other hand, feed must support animal health and welfare, ensure food safety and quality and, increasingly important, protect the environment and climate. Practically, a wide range of diseases occurs in both poultry and pigs, lower performance, impair animal welfare and lead to animal losses. Numerous studies carried out in recent years show that intestinal health is an essential factor for maintaining animal health. Intestinal health is influenced by nutritional factors, including dietary components, macronutrients, micronutrients and also feed additives. The composition and activity of the intestinal microbiota, the interaction with the intestinal mucosa and the associated immune system form an interacting triangle. The importance of this topic is increasingly seen and requires interdisciplinary research approaches. The lecture focuses on the role of feed for optimum intestinal function, a balanced intestinal microbiota, highlights the impact on the gut-associated immune system. This will be discussed for in pigs and poultry.

## The contribution of Specialty Feed Ingredients to sustainable animal farming / pivotal role in the fight against Antimicrobial Resistance

Joerg Seifert & Chiara Bellone de Grecis

Nutrition has a crucial function in animal performance as well as in the maintenance of good animal health and welfare status. Specialty Feed Ingredients used in livestock feed and in pet foods play a pivotal role. Among the different beneficial functions are:

- Ensuring optimal nutrition through an adequate supply of essential nutrients,
- Strengthening the digestive system to support immunity and vitality for better animal's defences against biological stressors,
- Improving living conditions by reducing ammonia exposure and emission for better air quality; reducing footpad lesions through dryer litter, resulting in an overall improvement of animal comfort,
- Stabilising physiological conditions to aid animals to cope with difficult stages inherent to their lifecycle, such as heat stress and post-weaning stress,
- Preserving feed as a measure to protect animals by preventing the exposure of feed to spoilage, toxins and to the colonization by undesirable micro-organisms (moulds, bacteria and their metabolites).

Maintaining animals in optimal health and, thus, with higher resilience to stressors, such as pathogenic micro-organisms, can prevent or limit the need for veterinary treatments, including the use of antibiotics.

### Author information

FEFANA - EU Association of Specialty Feed Ingredients and their Mixtures

## Studies on the mechanisms of action of phytogetic feed additives including Next-generation Sequencing (NGS)

Klaus Teichmann, Bertrand Grenier, Verity-Ann Sattler, Silvia Fibi, Nicole Reisinger, Suzana Ilic, Caroline Emsenhuber, Veronika Nagl, Theresa Schott, Elisabeth Mayer, Gerd Schatzmayr

Phytogetic feed additives (PFA) contain secondary metabolites known from plants, mostly medicinal plants or spices. While they are widely acknowledged as sensory additives, a small number of products has been approved so far as so-called zootechnical feed additives in the European Union. They gain more and more importance, because their activity goes beyond conferring flavour to feed. Such products provide additional benefits, e.g. by stabilizing the microbiota, enhancing feed digestibility, reducing the environmental impact of animal production, improving production performance, or animal welfare. Innovation in these areas is a crucial contribution to reducing the use of antibiotics in animal production. NGS provides fascinating new tools to increase our understanding of the mechanisms of action (MoA) of feed additives. Actually, different concepts about the MoA of growth promoting antibiotics are being discussed. The most widespread ones claim effects on the microbiota or on the immune system of the host animal.

In the current study, zootechnical PFA with proven records as natural growth promoters have been studied *in vitro* and *in vivo* in order to elucidate their possible MoA. The anti-inflammatory potential and effects on the immune system were studied in cell cultures and in feeding trials with broiler chicken and weaned piglets, including RNA-sequencing. Potential effects on the microbiota were studied in a feeding trial with weaned piglets using NGS of the 16S rRNA gene.

Cell culture experiments showed down-regulation of nuclear factor  $\kappa$ B and relevant genes involved in inflammatory processes, which are under its control, when extracts of the PFA were applied. In animal feeding trials, gene expression of pathways of the innate immune system was restricted, when animals were fed the PFA, and blood analyses showed lower levels of acute phase proteins. Significant changes to the microbial composition were apparent after application of an antibiotic, but not the PFA.

The main MoA of the studied PFA seems to involve immunomodulatory and anti-inflammatory pathways, rather than the microbiota and its composition. NGS is a valuable tool to create and test hypotheses on the MoA of feed additives and may assist in product development.

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## Undesirable substances in interspersed substrates – impact on animal and consumer health

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To provide a dry, hygienic environment, interspersed materials play an important role in animal welfare. These materials should also enable pigs to perform natural behaviour under intensive housing conditions. However, oral intake of such materials may affect animal health and the safety of food of animal origin if they contain heavy metals or dioxins (undesirable substances according to feed law). Although it is known, and in some cases even desired, that pigs consume the provided material, no legal regulation exists establishing maximum levels for heavy metals, trace elements and other undesirable substances in substrates dispersed in the pen.

The analysis of 71 materials (disinfectant powders [n=51], earth/peat [n=12], biochar [n=8]) revealed considerable amounts of trace elements, heavy metals and dioxins in some samples.

In order to answer the question, whether some materials would attract interest and could consequently be consumed by pigs, a behavioural study in a 4 x 6 factorial arrangement with camera-assisted analysis was conducted: Twelve pigs kept in 6 pens were tested for their preference for different combinations of disinfectant powder, peat, biochar and straw. Video recordings were analyzed focusing on frequency and total time of pigs exploring the materials. The highest preference was determined for earth/peat and biochar, followed by straw and disinfectant powder. Furthermore, analysis of pigs' faeces receiving the material combination peat-straw for long chain n-alkanes (in a range of 25 to 36 carbon atoms) and humic acids, naturally occurring in the provided materials, confirmed an oral uptake of peat and straw by pigs. Faeces from pigs receiving the combination disinfectant powder-straw had higher concentration of HCl-insoluble ash as compared with faeces of pigs receiving no treatment.

Consequently, due to a considerable consumption by the animal, high levels of undesirable substances and trace elements in these substrates might exceed the maximum level in the daily ration and a possible transfer into food of animal origin has to be considered. Future studies will address the quantification of the actual ingested amount of interspersed substrates by pigs as first step towards the estimation of the transfer of undesirable substances from these substrates into the food chain.

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## European Feed Microbiology Organization – Promoter of microbiological feed quality

Manuela Zadavec, Beatrix Konemann, Jacqueline Wolfram, Henriette Mietke-Hofmann

It is well known that the content of bacteria and moulds affects the quality of feed and consequently animal health and production. The EFMO (European Feed Microbiology Organization) emerged from the IAG (International Analytical Group, Feed Microbiology), and was renamed and founded as an association on 27 May 1997 at the first meeting in Karlsruhe. The EFMO gathers European feed microbiologists and helps them implement methods for the evaluation of microbiological quality of feed. The main goal of the EFMO is to implement the VDLUFA method for feed microbiological quality estimation. The method is based on a systematic approach, which categorizes feed into categories from 1 to 4 according to bacteria, moulds and yeasts content, and their ecology and hazard signification. Classical microbiological methods are used to determinate the content of microorganisms. The VDLUFA method was created after monitoring the microorganisms contents in feedstuffs in Germany, Austria and Switzerland. The process started around 1970 in the organization of the Association of the German Agricultural Analytic and Research Institutes (VDLUFA). This monitoring resulted in the publishing of a unified method for the microbiological feed quality in 1981. Using that method a huge study was arranged where the base orientation values were established, according to which feeds are evaluated and categorized. Except for the help in the VDLUFA method implementation, the EFMO serves for the exchange of experience and knowledge between researchers and laboratories. Additionally, since 1980 the EFMO has organized proficiency testing in the framework of samples which are analysed simultaneously by 10 to 20 laboratories from Germany, Austria, Switzerland, Slovenia, Hungary, Portugal, France and Croatia. Considering that none of the European regulations proscribes the values for feed microbiological quality, the mission of the EFMO is to promote the importance of the microbiological quality of feed on animal health, and additionally, the tool for control in feed production and in its storage.

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## Official Controls and Accredited Third Party Certification; Working together to secure the feed supply chain

Emmanouil Geneiatakis, FAMI-QS

The quality and safety of feed for animals intended for human consumption will have a direct impact on the quality and safety of the food produced from these animals. Given the anticipated growth of the world's population to around 9 billion people by 2050, and the associated higher demand for animal proteins, it is vital that the feed industry, as well as the wider agricultural chain, can meet this challenge in a sustainable and safe way.

Third-Party Certification (TPC) and particular feed schemes are becoming an important feature of the global feed chain. Nowadays, Feed Business Operators' operational activities take place in a rather complex legal and business environment. Today we have moved from local sourcing to global sourcing. This affects the level of controls which are required. Although TPC is voluntary, it is an important requirement for a Feed Business Operator to gain market access and to communicate all aspects related to the safety of the feed ingredient, in a uniform language.

The implementation of a feed safety management system is not just paperwork or a commercial activity for the Certification Bodies. Most companies fail to implement properly a feed safety management system because either they implement it under pressure by their clients or they consider it as a bureaucratic approach. It is important that the feed sector develops a better understanding on the feed safety culture. Third-Party Certification can further contribute to this.

Besides its market value, a credible Third-Party Certification could also be a useful tool in the hands of the competent authorities regarding, e.g. planning of official controls or "granting" market access to products or operators. Third-Party Certification is not meant to substitute the responsibilities of competent authority but is increasingly recognised by a number of them as an important element, which not only supports their enforcement role, but can also help in setting state-of-the-art practices at global level and as such facilitate understanding and exchanges at international level.

### Author information

Mr. Emmanouil Geneiatakis is working with FAMI-QS Since 2009. He is major in Agribusiness with a Master in Applied Geography and Spatial Development.

## Session 5 Sustainability and Circular Economy

### Keynote: FEFAC Charter 2030: A comprehensive view on sustainable feed production

Asbjørn Børsting

In September 2020 FEFAC launched its Feed Sustainability Charter 2030. The Charter, contains five ambitions which intend to address all relevant aspects of sustainable feed production; climate neutrality, resource & nutrient efficiency, responsible sourcing, enhancing animal health & welfare through animal nutrition and enhancing the socio-economic environment.

In June 2021 FEFAC will publish its first Progress Report, outlining the activities and achievements in the context of the Feed Sustainability Charter since September 2020, including a series of webinars, the release of the FEFAC Soy Sourcing Guidelines 2021 and assessments on the use of non-food feed ingredients and EU-aggregate averages of feed conversion ratios of farm animals.

The FEFAC Feed Sustainability Charter is intended to inspire and facilitate the development of Sustainability Charters, Roadmaps and Agendas by FEFAC members at national level, where the most concrete commitments to action will be made.

## Effects of confectionary or bakery former food products as cereal substitute on growth performance in post-weaning piglets

Pinotti L, Luciano A, Comi M, Fumagalli F, Rovere N, Manoni M., Ottoboni M

Exploiting leftover streams is one way to decrease the environmental impact on the livestock sector.

In particular, former foodstuffs products (FFPs) have a great potential to be used as alternative feed ingredients in livestock production. FFPs are mainly characterized by leftovers from the bakery and confectionary industry, such as bread, biscuits, snacks and broken pasta, that make them quite similar to cereals. Accordingly, in this study, conventional cereal grains have been partially replaced by two category of FFPs (sweet or salty) in post-weaning piglet's diets in order to investigate the effects of these materials on pig growth performance and digestibility.

Briefly, 36 weaned female pigs (28 d of life,  $6.70 \pm 1.07$ kg) were fed 1 of the 3 experimental diets: 1) Control diet (CRT), 0% FFPs; 2) confectionary FFPs diet (FFP sweet) in which 30% of cereals were substituted by 30% of confectionary FFPs; 3) bakery FFPs diet (FFP salty) in which 30% of cereals were substituted by 30% of bakery/snack FFPs. The diets were iso-energetic (15.3 MJ/kg DM) and iso-nitrogenous (19% CP DM), and met NRC (2012) requirements. The trial lasted 42 d. Individual pig body weight (BW) and feed intake (FI) were measured weekly. During the experiment, stool samples were also collected for determining apparent total tract digestibility (ATTD). In addition, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were be calculated. At the end of the experiment, no differences in BW were observed between groups ( $P > 0.05$ ). The results revealed that ATTD values was higher ( $P < 0.05$ ) for FFP salty > FFP sweet that was comparable to CTR diet. Overall ADG, ADFI, and FCR were not affected by any dietary treatments. Taken together, these results suggest that the use of FFPs up to a level of 30% in post-weaning diets has no detrimental effects on pig growth performance, although more studies are needed to confirm these results.

The present work has been done ion the frame of the following project: SUS-FEED-funded by the Fondazione Cariplo, call "Economia circolare: ricerca per un futuro sostenibile".

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## Multi-omics analysis of Atlantic salmon liver tissue after exposure to pirimiphos-methyl

Rasinger J.D., Lie K., Bernhard A., Ørnstrud R., Sanden M., Berntssen M.H.G.

To increase the sustainability of aquaculture, ingredients other than fish meal and fish oil are increasingly being used in feed for carnivorous farmed fish. This introduces contaminants into the food chain, which earlier were not directly associated with fish farming. For example, in wide-scope chemical screening studies, the organophosphate pesticide pirimiphos-methyl (PM-m) was reported to be present in plant-substituted commercially produced Atlantic salmon feed. The present study used high throughput multi-omics and bioinformatics tools to describe dose response relationships and mechanisms of action of PM-m exposure in farmed Atlantic salmon (*Salmo salar*). In an in vivo trial, salmon were exposed to graded levels of PM-m spiked feeds (0, 0.35, 1.5, 5, and 22 mg kg<sup>-1</sup>) in triplicate for three months. A total of 45 salmon liver samples (n=15/group) were prepared for and subjected to RNAseq and high-resolution mass spectrometry analyses. RNAseq data revealed that out of a total of 28640 transcripts identified, 2244 displayed significant ( $p < 0.05$ ) changes in expression levels. Out of 4613 identified proteins, 2570 were eligible for further statistical analysis; the abundances of 255 of these proteins were found to be significantly ( $p < 0.05$ ) altered. Direct comparison of RNAseq and proteomics data revealed an overlap of 59 features. Tentative bioinformatic analyses highlighted these features to be linked to disruptions of proteasome activity and mitochondrial function, respectively; both implicated to affect hepatic lipid metabolism. Detailed multi-omics data integration and work regarding the identification of altered molecular pathways affected by PM-m is still on-going. When complete, the data obtained in the present work will contribute to the mechanistic understanding of the hepatotoxicity of PM-m and provide molecular explanations for the effects pesticide contaminated feed elicit in farmed Atlantic salmon in vivo.

### Author information

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## Extraction of functional nutritive derivatives from unicellular organisms by novel technologies for sustainable production of animal feed

Miladinovic, Dejan; Svihus, Birger; Odd Ivar, Lekang; Salas-Bringas, Carlos

Sustainable feed manufacturing is important for sustainable farming.

Diversification of the expensive protein sources is one of the most crucial aspects for sustainable feed manufacturing.

Using unicellular organisms to substitute the fishmeal could be considerably used in the future.

Currently, most feed ingredients are treated at high temperatures.

However, treating certain feed ingredients during upstream and downstream processes must be done at low temperatures.

In such way, the overcooking and undercooking of valuable nutrients could be avoided.

In this review, different Commercial (extrusion and pelleting) and novel (pulsed electric fields, high pressure Processing and steam explosion) technologies and their applications will be described along with their potential for extracting functional nutritive derivatives for better animal health and nutrition.

### Author information

Head of Centre for Feed Technology, Norwegian University of Life Sciences (NMBU).

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## Connecting agri-food supply chains through insect farming: upcycling underused materials into animal feed ingredients

Christophe Derrien

Insect farming is among the most efficient farming practices, upcycling underutilised resources into higher-value protein and lipids that have multiple uses in both food and feed chains. Building on the ability of insect larvae to biotransform a wide range of materials, the European insect sector aims at making a bolder contribution towards EU's target to halve food waste by 2030. According to IPIFF's estimations, up to a third of the food waste generated in the European Union could be suitable with insect farming practices (IPIFF members estimate that circa 20 million tonnes of former foodstuffs could be used for the food/feed chains, with several tonnes of catering waste which could be used in technical applications). The incorporation of such products - before they become waste - could play a key role in improving EU's self-sufficiency in terms of protein-rich feed materials. The objective of this presentation is to present an overview of the legislative opportunities, but also the regulatory roadmap of the European insect sector with regards to new substrates for insect farming.

The advantage of the circular practices implemented in insect farming make such systems less and less dependent on finite natural resources. The use of underused resources, such as food products intended for human consumption presents a viable source of nutrients for insect farming. Such goods - referred to as 'former foodstuffs' - have equivalent characteristics to other presently used materials used in animal feed, such as cereals for example. We are confident that by 2030, the European insect sector will remain a committed partner in achieving EU's target to halve food waste, by upcycling former foodstuffs into valuable ingredients for the agri-food chains. Redirecting a wider share of the former foodstuffs that are not authorised in animal feed towards insect farms could play an important role in improving EU's self-sufficiency in terms of protein-rich feed materials.

Keywords: insect farming, insect substrates, former foodstuffs, alternative feed ingredients, EU feed legislation.

### Author information

IPIFF is an EU non-profit organisation which represents the interests of the insect production sector towards EU policymakers, European stakeholders and citizens.



## 101 IAG Ring Test - A world-wide Interlaboratory Comparison

Gerhard Liftinger, Magdalena Wagner, Irmengard Strnad

The IAG (International Analytical Group, Section Feed Analysis) organizes a yearly world-wide interlaboratory comparison program for feedingstuffs based on EN ISO 17043. Purpose and aim of this proficiency test is to demonstrate the laboratories' performance in feed analysis, without prescribing any methods of analysis or minimum number of analysed parameters. This type of organization provides the opportunity to compare the performance of chemical methods and screening methods.

The proficiency test comprises four samples (each 300-700g) for various feed analyses and two additional samples for which only the moisture content is determined.

The range of parameters offered is very wide including ingredients (e.g. XP, XL, XF, XA), fibre fractions, elements, heavy metals and feed additives like amino acids, phytase and vitamins. Generally, all samples are commercially available unground products (grass meal pellets, mixed feed and mineral feed), but one of the samples is ground compound feed spiked with special components such as MHA, Hg, Cr, Ni, antioxidants or organic acids.

Statistical evaluation of the results is carried out according to ISO 13528. Outliers are removed and mean, reproducibility, relative reproducibility and range of tolerance are reported for each parameter. For the assessment of the laboratory z-score and recovery rate is specified.

In the year 2020 54 laboratories from 21 countries participated in this proficiency test and over 1600 results were statistically evaluated.

Each laboratory is given the opportunity to attend an online meeting to discuss the final report in detail whether there were any analytical problems and which parameters had good or bad results. Participation in this ring test is an important quality assurance measure for the laboratories to get the possibility to determine the accuracy of their results, to verify the technical expertise of their staff and to assess the functional capability of their laboratory equipment.

More detailed information is given on following website:

<https://www.ages.at/service/service-tierernaehrung/futtermittel/ringversuche/iag/>

# Posters

## 102 Development and validation method for detection and determination of formaldehyde in compound feeds and silage by HPLC-DAD detector after precolumn derivatization

Ewelina Patyra, Krzysztof Kwiatek

Formaldehyde had been used for many years as a feed additive as an additive from the group of technological additives "and the functional group" preservatives. The use of formaldehyde as a feed additive was limited to silage in order to stop fermentation processes and skimmed milk for piglets (Council Directive 70/524 / EEC, 2004 / C50 / 01). On 7th February 2018, Commission Implementing Regulation (EU) 2018/183 was issued refusing to authorize the use of formaldehyde as a feed additive belonging to the functional groups 'preservatives' and 'hygiene improving substances'.

For the purpose of formaldehyde control in feed and silage, a simple and robust method is proposed for the analysis of formaldehyde. The method is based on the determination of the fluorescent product of chemical reaction between formaldehyde and 2,4-dinitrophenylhydrazine. A 3 g feed or silage were extracted with Milli-Q water with phosphoric acid and next formaldehyde was derivatized with the use 2,4-dinitrophenylhydrazine in acetonitrile solution. The purified extract was separated on Zorbax Eclipse XDB C18 column and detection was carried out at 360 nm. This method provided average recoveries of 90.6% to 102.2%, with CVs of 2.6% to 6.4% for feed and from 91.3% to 108.7% with CVs of 1.1% to 4.1% for silage in the ranged of 50 to 1000 mg/kg feeds and silage.

This study investigated the level of formaldehyde in feed and silage samples cattle and poultry farms and feed mills from different region of Poland. In 2018 and 2019 total of 97 samples (samples of alfalfa, corn and grass silages, wet beet pulp and feeds) were analysed. Our study showed that only 1 (1.03%) tested feed sample was contaminating formaldehyde. Our study showed that feed and silage produced in Poland was free from formaldehyde used. Good manufacturing practice and good hygiene practice allows for a low level of contamination of feed and silage of Salmonella, so that formaldehyde is not used as a preservative for feed and silage in Poland.

Analytical chemistry, antibacterial substances, feed, faeces, feed additives, HPLC, DAD, FLD, MS, solid phase extraction, QuEChERS, validation

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## 103 LC-MS/MS method for the quantification of tiamulin, trimethoprim, tylosin, sulfadiazine and sulfamethazine in medicated feed

Ewelina Patyra<sup>1</sup>, Carolina Nebot<sup>2</sup>, Rosa Elvira Gavilán<sup>2</sup>, Krzysztof Kwiatek<sup>1</sup>, Alberto Cepeda<sup>2</sup>

Since 2006, when the European Commission banned the use of antibiotic growth promoters, increased use of medicated feedingstuffs has been observed in animal production. Medicated feed is a homogenous mixture of feed and veterinary medicinal product(s) and should only be manufactured with authorised veterinary drugs. In order to ensure a particularly prudent use of medicated feed for food-producing animals compliance with the withdrawal period and record-keeping by the animal holder keeper, where appropriate, should be provided. Taken into consideration the last EU report on the use of antimicrobials, their common use in of premixes and the absence of analytical methods validated according to the EU legislation, this paper reports the development of a selective and sensitive new method for simultaneously analysing five antimicrobial agents (tiamulin, trimethoprim, tylosin, sulfadiazine and sulfamethazine) in one single extraction protocol in medicated feed for different animal species (poultry, swine and cattle) using only liquid extraction and liquid chromatography with tandem mass spectrometry. Antibacterial substances were extracted with the use 0.1% formic acid in acetonitrile, next centrifuged, diluted in Milli-Q water, filtered and analysed by high performance liquid chromatography coupled to tandem mass spectrometry. The separation of the analytes was performed on a biphenyl column with a gradient of 0.1% formic acid in acetonitrile and 0.1% formic acid in Milli-Q water. Quantitative validation was done in accordance with the guidelines laid down in European Commission Decision 2002/657/EC. Method performances were evaluated by the following parameters: linearity ( $R^2 < 0.99$ ), precision (repeatability  $< 14\%$  and within-laboratory reproducibility  $< 24\%$ ), recovery (73.58 – 115.21%), sensitivity, limit of detection (LOD), limit of quantification (LOQ), selectivity and expanded measurement uncertainty ( $k=2$ ). The validated method was successfully applied to the medicated feeds obtained from the interlaboratory studies and feed manufactures from Spain. In these samples tiamulin, tylosin and sulfamethazine were detected at the concentration levels declared by the manufacturers. The developed method can therefore be successfully used to routinely control the content and homogeneity of these antibacterial substances in medicated feed.

Analytical chemistry, antibacterial substances, feed, faeces, feed additives, HPLC, DAD, FLD, MS, solid phase extraction, QuEChERS, validation

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## 104 Emulsions as antimicrobial delivery system to inactivate salmonella in feed

Hilde Wouters, Liesbet Thijs, Inge Peeters, Ingrid Somers, Filip Nuyens

Surfactant micelles were studied as a delivery system for essential oils to better reach Salmonella in animal feed. In natural environments, bacterial cells occur rather as multicellular aggregates than as planktonic cells. Aggregated cells are metabolically less active and more resistant to antimicrobials. Essential oils exhibit antimicrobial activity against feed pathogens. Most reported research with essential oils however, has focused on the antimicrobial activity of these compounds against planktonic cultures. Determination of the minimal inhibitory concentration (MIC) of six essential oils was performed against planktonic cells of *S. typhimurium* ATCC 14028 and *S. infantis* (a feed isolate). Carvacrol showed the highest anti-Salmonella activity with an MIC of 200 ppm. Even a lower MIC of 100 ppm was obtained with a combination of thymol and carvacrol.

Six emulsion formulas were prepared by addition of carvacrol (and thymol), organic acids as co-surfactant and surfactants into an aqueous solution. *S. typhimurium* ATCC 14028 was grown as biofilms in agar plates. The biofilms were treated with the different formulas. A blend of organic acids (formic/propionic acid) was included as a positive control. Visualization of viable cells in the biofilms was performed by using resazurin. Formula 2, dosed at an equivalent of 200 ppm carvacrol, resulted in a reduction of ~3-log CFU biofilm mass within 4 h of exposure time.

A broiler diet was artificially contaminated with *S. typhimurium* ATCC 14028 and then treated with the emulsions or organic acid blend. The first results revealed that formula 2 applied at 5 kg/T (50 ppm carvacrol) and 40 kg/T feed (400 ppm carvacrol), reduced Salmonella counts by 0.3 log (50% reduction) and by 0.6 log (72% reduction), respectively. Compared to the organic acid blend (5 kg/T), a further reduction of 32% and 62% could be observed with 5 kg/T and 40 kg/T formula 2, respectively. In conclusion, a clear antimicrobial efficacy of the emulsions against Salmonella was found in both models. Although bacterial cell injury was statistically significant ( $p < 0.05$ ), none of the final reductions achieved the desired target of 1-log in the feed. In future research, this new strategy will be further optimized.

### Author information

Innovation project leader with focus on feed safety projects

## 105 Microbiological safety of insect feed

Magdalena Goldsztejn, Tomasz Grenda, Nina Kozieł, Magdalena Grabczak, Małgorzata Mazur, Beata Król, Zbigniew Sieradzki, Krzysztof Kwiatek

### Objective

It is considered as a novel idea to include insects in animal feed, specifically insect protein, however the microbiological safety of them is under examination and discussion. The potential, biological threat could be associated with occurrence of pathogens originated from soil, carcasses in potentially high number.

Microbiological quality of insect feed is still underestimated and should be taken into consideration.

The aim of the study was examination of insect feed samples collected in Poland towards microbiological contaminants evaluation.

### Material and methods

The analyses were carried out on 36 samples of insect feed. Samples were examined towards general number of microorganisms (according to PN-EN ISO 4833), Enterobacteriaceae enumeration (ISO 21528-2:2017), Yeast and moulds enumeration (PN – R – 64791:1994), *Salmonella* spp. occurrence (ISO 6579-1:2017), *Clostridium* sp. occurrence (PN – R – 64791:1994), *Enterococcus* spp. (EN 15788:2009).

### Results and discussion

Results showed the high number of microorganism ranged between 102 in 1/36 samples (3%) to 106cfu/g in 4/36 samples (11%). Clostridia were found in 30/36 (83%) of samples. We noticed high contamination with *Clostridium* sp. at the level of 105cfu/g in 3/36 samples (8%) whilst contamination of standard feed material usually does not exceed the level of 104cfu in 0.5% of samples. Enterobacteriaceae number varied between 101cfu in 8/36 samples (22%) to 105cfu in 1/36 sample, when contamination level of standard animal meals usually does not exceed the level of 104 cfu/g. The high number of samples were contaminated with enterococci 28/36 (78%) and number of this bacteria ranged between 102 in 11/36 samples (31%). Yeast and moulds number was estimated at the level between 101cfu in 8/36 (22%) to 105cfu in 1/36 (3%) samples. The conducted analyses indicated on high microbiological contamination and should be continue in order to estimate potential risk for animal and human health.

### Author information

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## 106 *Clostridium botulinum* occurrence in insect feed

Tomasz Grenda, Magdalena Goldsztejn, Nina Kozieł, Ewelina Patyra, Zbigniew Osiński, Krzysztof Kwiatek

### Objective

*Clostridium botulinum* is the species of microorganisms able to produce the most potent toxins in environment. Larvae of Insects are considered as vectors of *C. botulinum* strains originated from soil and carcasses. In literature several reports are described on avian and probable cattle botulism cases caused by larvae of necrophagous flies. The aim of this study was screening analysis of Insect feed produced in Poland towards *C. botulinum* and phenotypically similar strains occurrence.

### Materials and methods

The analyses were conducted on 36 samples of insect feed. Samples were examined towards occurrence of strains phenotypically similar to *C. botulinum* species. Subsequently, liquid cultures of insects feed inocula and isolates grown on Willis- Hobbs and FAA differential media were subjected to screening analysis with using real-time PCR method based on *ntnh* gene detection determining non-hemagglutinin component of botulinum protoxin, common in all *C. botulinum* toxin types. Real – time PCR method was previously validated and compared to mouse bioassay (MBA) after individual permission obtained from II Local Etic Commission in Lublin (Poland).

### Results and discussion

The strains phenotypically similar to *C. botulinum* species with lipolytic properties and subterminal spores location were isolated from 6 samples (17%) of examined insect feed. None of them possessed *ntnh* genes. However DNA isolated from one liquid culture in TPGY broth (one of which mentioned strains were isolated from) showed the presence of *ntnh* gene indicated on possible occurrence of *C. botulinum*. *Clostridium botulinum* toxigenicity determines possible horizontal gene transfer or lysogenic bacteriophages occurrence in environment which could be lost during isolation process. High heterogeneity of this microorganism and occurrence of phenotypically similar strains make laboratory detection complicated and labour. Occurrence of possible *C. botulinum* strains should be taken into consideration in risk assessment of possible botulism cases occurrence in animals after feeding with insect feed. This study was financially supported by the National Centre for Research and Development 2018–2020 in the project frame GOSPOSTRATEG 1/385141/16/NCBIR/2018.

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## 107 HPLC-FLD method for the determination of ergot alkaloids in feeds

Ewelina Kowalczyk, Aleksandra Grelik, Krzysztof Kwiatek

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Ergot is the sclerotium of parasitic ascomycetes that belongs to the *Claviceps* genus.

The most widespread *Claviceps* species in Europe is *Claviceps purpurea*, which is known to infect more than 400 plant species including rye, wheat, triticale, barley and oats. The sclerotia are harvested with the grains and may result in contamination of cereal products and feeds. The health concern connected with the consumption of sclerotia is the fact that they produce toxic secondary metabolites ergot alkaloids (EAs). Generally, ergot alkaloids can cause a broad spectrum of adverse health effect leading mainly to the ergotism disease. Ergotism remains an important veterinary problem, especially in cattle, horses, sheep, pigs and chicken. In 2012 The Commission Recommendation on the monitoring of the presence of ergot alkaloids in feed and food was published. According to the Recommendation, it is necessary to generate more data on the presence of ergot alkaloids, not only in ungrounded cereals but also in cereal products and compound feed (European Commission 2012/154/EU). Thus, it is important to develop reliable methods for the determination of ergot alkaloids that could be introduced to laboratory practice and effectively implemented in the Official Control of Feedingstuffs. The aim of this study was the development of a simple analytical method based on high performance liquid chromatography combined with fluorescence detection (HPLC-FLD) enabling determination of 12 ergot alkaloids: ergometrine, ergotamine, ergocornine, ergocryptine, ergocristine, ergosine and their corresponding epimers in feeds. Ergot alkaloids are extracted with acetonitrile. The extract is subsequently purified with PSA and activated carbon. Cleaned extract is evaporated in the nitrogen stream and the residue is reconstituted in mixture of acetonitrile and 1 mM ammonium carbonate, passed through a nylon syringe filter and subjected to the instrumental analysis. Ergot alkaloids are analysed with HPLC-FLD instrument. Developed method was validated according to the Commission Decision 2002/657/EC. Parameters like method linearity, limits of detection (LODs), limits of quantification (LOQs), repeatability, within-laboratory reproducibility, recovery, specificity were evaluated. All determined parameters fulfilled the document recommendation, confirming the reliability of the developed method.

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## 108 Surfactants as cofactors of antimicrobials

Liesbet Thijs, Caroline Paulussen and Sten Janssen

Salmonella remains an important foodborne pathogen with a significant health and economic impact. The pathogen can persist for long times in a wide range of feeds due to its specific physiology and its ability to form a biofilm, which can be defined as a complex aggregate of bacterial cells embedded in a matrix. Organic acids are commonly used in the feed industry to control Salmonella on feed. A more efficient kill off by thermal and/or chemical treatment could be achieved by adding surfactants which are able to disrupt the matrix. In a first study, 5 surfactants were selected based on their characteristics and added in different ratios to achieve synergistic antimicrobial effects with organic acids on feed. The challenge in this research, however, is the stability of the surfactant mixture as a clear and stable emulsion to fully exploit the potential of the surfactants. Only a specific ratio of the surfactants appeared to be stable and remained stable when it was diluted further in water. In the field, this diluted form is then sprayed onto feed to optimize the contact between product and feed. In a second part, a biofilm disruption model was developed to demonstrate the efficacy of the surfactants in combination with organic acids. A robust biofilm was grown in a 24 well plate at room temperature for 24h in tryptic soy broth. After incubation, the biofilm was stained with crystal violet. In this test, different surfactant bases with the 5 selected surfactants were prepared and added to the organic acid blend. The efficacy of the prototypes in disrupting the biofilm was evaluated visually as the cells detached from the plate. The surfactant blend which performed superior in the first study was also the most efficient in disrupting the biofilm, demonstrating the importance of stable products. This study demonstrates the importance of surfactants to guarantee a safe feed to the customer.

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## 109 Studies on the transfer of Quinolizidine Alkaloids from blue sweet lupins (*Lupinus angustifolius*) into the milk of dairy cows

Hildburg Fry, Fenja Klevenhusen, Mandy Schwieters, Kerstin Mietle, Monika Lahrssen-Wiederholt, Robert Pieper

Due to their high crude protein content and nutritional value, the seeds of sweet lupin varieties may serve as alternative protein sources in diets for pigs, poultry and cattle. However, among other antinutritional factors, even sweet lupins still contain low amounts of Quinolizidine Alkaloids (QA), but the QA concentration may vary due to different factors e.g. geographical origin, selective pressure or from year to year. At higher concentrations some QA are toxic for humans and farm animals, respectively. Thus, besides putative adverse effects of lupins with higher QA concentrations on animal health, a transfer into food of animal origin may have consequences for food safety as well.

In a first step an existing LC-MS/MS method for the determination of QA in lupin seed was optimised and validated for compound feed with lower QA concentrations. In a second step a LC-MS/MS method for the determination of low concentrations of the QA lupanine, sparteine, lupinine, 13-hydroxylupanine, isolupanine, angustifoline and anagyrene in lupin protein containing food and in cow milk was established and validated. First investigations of cow milk samples suggest a possible transfer of QA into milk.

To answer the question of a possible transfer of QA from the feed into food of animal origin (milk), a transfer study was initiated. Blue sweet lupins were fed in two concentrations in a total-mixed ration (TMR) replacing rapeseed meal to three lactating dairy cows. Milk samples will be continuously collected and analysed for their QA concentrations allowing the calculation of the transfer rates of individual QA.

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## 110 Physiologically based toxicokinetic models with in silico predicted partition coefficients to predict the transfer of tetrachlorodibenzo-p-dioxin from contaminated feed into adipose tissue and liver of growing pigs.

Savateeva D., Buschhardt T., Pieper R., Lahrssen-Wiederholt M., Numata J.

Tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous, toxic, persistent and bioaccumulative organic pollutant. TCDD can potentially enter the food chain through contaminated food of animal origin as a consequence of the presence of TCDD as an undesirable substance in feed. Prediction of the TCDD transfer from feed into animal products is thus important for human health risk assessment. Here, we present several physiologically based toxicokinetic (PBTK) models of TCDD transfer from contaminated feed into growing pigs (*Sus scrofa*) to evaluate absorption, distribution, metabolism and excretion of the contaminant. The modelled animals were exposed to various doses of TCDD. We test the consequences of explicit dose-dependent absorption (DDA) versus the indirect effects of a self-induced liver metabolism (SIM). In silico predicted partition coefficients were implemented as model parameters, as they are a useful alternative to in vivo experiments for kinetic modelling. The DDA and SIM models showed similar fit to experimental data, although currently it is not possible to unequivocally make statement on a mechanistic preference. The performance of both toxicokinetic models was successfully evaluated using the 1999 Belgian case of contaminated fats for feeding. In combination with toxicokinetic models of other dioxin congeners, they can be used to formulate maximum allowance levels of dioxins in feedstuffs for pigs.

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## 111 Investigations on the transfer of non-dioxin like Polychlorinated Biphenyls (ndl-PCB) from feed into meat of broilers and eggs of laying hens

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Concentrations of non-dioxin-like polychlorinated biphenyls (ndl-PCBs) higher than the legal maximum levels were detected in eggs and meat of turkey and chicken. In some samples, maximum level of food of animal origin was exceeded even though the feed did not exceed the legal maximum permitted levels. At present, limited information exists on the transfer of individual ndl-PCB congeners from feed into food of animal origin. Improved knowledge on the transfer of congeners from targeted studies may allow the development of predictive tools for management purposes. In a follow-up study, contaminated eggs were analyzed in order to identify the time dependent occurrence and decline of indicator ndl-PCB and individual congeners in eggs. A different distribution pattern of the ndl-PCB compared with a previously published toxicokinetic model for dioxins and PCB was observed. In order to get more data for the modelling of the distribution pattern of ndl-PCB congeners in animals, two transfer studies in poultry were conducted. In one study, 48 broilers divided into 6 groups of 8 animals each were fed with ndl-PCB contaminated feed for different time periods before slaughter. Indicator ndl-PCBs in pectoral muscle were determined. In the second study, contaminated feed was given to two groups of laying hens (n=15 each) for 30 and 60 days, respectively and hens received non-contaminated feed afterwards during a 3-month washout period. Egg samples were taken at regular intervals and analyzed for total and individual ndl-PCB congeners

The data allows an improvement of existing toxicokinetic models for the transfer of ndl-PCB and individual congeners from feed in food of animal origin.

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## 112 Mycotoxins are ubiquitous contaminants in animal feed

Christiane Gruber-Dorninger, Wolfgang Schweiger, Timothy Jenkins and Gerd Schatzmayr

Fungi such as *Fusarium* spp. and *Aspergillus* spp. infest crop plants in the field or agricultural commodities during storage. These fungi produce mycotoxins, i.e. toxic secondary metabolites. Mycotoxins can exert toxic effects in animals, thereby compromising animal wellbeing and productivity. Furthermore, mycotoxins may be transferred into animal products. Therefore, mycotoxin occurrence in animal feed should be monitored closely. To this end, we performed an extensive 13-year survey investigating global mycotoxin occurrence in feed. More than 130,000 samples of feed and feed raw materials (e.g. maize, wheat, soybean) were collected from 113 countries from 2008 to 2020. Concentrations of aflatoxin B1, zearalenone, fumonisins, ochratoxin A, deoxynivalenol and T-2 toxin were analyzed. We found that the vast majority of the samples (89%) was contaminated with at least one mycotoxin. Prevalence and concentrations of individual mycotoxins varied between regions according to differences in climate and other factors, resulting in region-specific challenges to feed safety. For example, while the majority of samples from other regions complied with the EU maximum level for the carcinogen aflatoxin B1, significant fractions of samples from South Asia, Sub-Saharan Africa and Southeast Asia exceeded this maximum level, suggesting a risk for animal and human health. In many regions, mycotoxin concentrations in maize showed a pronounced year-to-year variation. In several cases, this variation could be correlated with weather during sensitive periods of grain development. For example, relatively high concentrations of deoxynivalenol and zearalenone detected in maize harvested in 2014 in Central and Southern Europe could be correlated with high levels of rainfall in July of that year, when maize plants were in the silking stage. The majority of the samples analyzed in this survey (67%) were co-contaminated with at least two mycotoxins, underlining the necessity for more research on the toxicological effect of mycotoxin mixtures. In conclusion, mycotoxins are ubiquitous contaminants in animal feed and an ongoing challenge to global feed safety.

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## 113 Sensitivity of Atlantic salmon to dietary pirimiphos-methyl exposure in plant-based feeds

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The use of plant-based feeds has introduced pesticides that have previously not been associated with seawater farming of fish species such as Atlantic salmon. Earlier wide-scope chemical screening showed that the organophosphate (OP) pirimiphos-methyl (PM-m), is one of the most prevalent pesticides present in commercially produced Atlantic salmon feed. Information on the safe upper limit of PM-m in Atlantic salmon feeds with regards to possible adverse effect on fish health is lacking. Atlantic salmon (132 ± 25g) were fed graded levels of PM-m spiked feeds (0, 0.35, 1.5, 5, and 22 mg kg<sup>-1</sup>) in triplicate for 3 months. Adverse effects were assessed on OP target toxic responses such as plasma choline esterase (ChE), and secondary toxic responses such as lipid metabolism and oxidative stress, as well as general adverse effect parameters (plasma biochemistry, hematology, and growth). Safe limits were set by model-fitting the effect data in a dose-response (lower bound) benchmark dose (BMDL) regression analysis. Fish fed 1.5 mg kg<sup>-1</sup> and higher had a significant ( $p < 0.05$ ) dose-dependent growth reduction, oxidative stress as seen from reduced glutathione ratio, liver damage as seen from plasma alkaline phosphatase, and reduced sum neutral and TAG liver lipids. Inhibition of ChE was observed in fish fed 5 mg kg<sup>-1</sup> and above. Disturbance in phospholipid (PL) fatty acid composition and reduced level of liver phosphatidyl choline (PC) occurred at a lower exposure level (0.35 and 1.5 mg kg<sup>-1</sup>), however this was not dose-responsive as at higher exposure levels (5 and 22 mg kg<sup>-1</sup>), no significant differences were observed. A safe feed limit (as BMDL) for dietary PM-m was set at 0.14-0.46 mg kg<sup>-1</sup> ww feed (daily dose 0.49- 1.62 ng kg BW<sup>-1</sup> day<sup>-1</sup>), based on inhibited growth, ChE, glutathione ratio, and sum neutral and TAG liver lipids. When including an uncertainty factor (UF) of five for inter-species variability and extrapolation to chronic exposure, the safe limits are 0.028-0.092 mg kg<sup>-1</sup>, which is lower than the highest PM-m levels observed in commercial salmon feeds (0.038 mg kg<sup>-1</sup>). Thus indicating a potential risk for disturbance in lipid metabolism when Atlantic salmon is fed plant-based feeds.

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## 114 Fluorine occurrence in animal feed

Maria Cesarina Abete, Paola Brizio, Caterina Stella, Anna Riva, Sabina Pederiva, Stefania Squadrone.

Fluorine is an essential element for animal and humans, in fact low levels in fluorine resulted in growth retardation and impaired fertility. Excessive exposure instead is associated with dental and skeleton abnormalities, as well as nephrotoxicity (EFSA, 2004). Fluorine element occurs in different chemical forms, but in environment, food, water and plants it is mainly present in its ionic form, fluoride. Moreover, fluorides are natural components in phosphate and super-phosphate fertilizers used in agriculture and fluoride-bearing rock phosphate is used in mineral supplements for livestock.

Commission regulation (EU) No 186/2015 amending Directive 2002/32/EC of the European Parliament and of the Council set the maximum levels of fluorine in animal feed. Then, the National Reference Centre for the Surveillance and Monitoring of Animal Feed (CReAA) developed a method for the detection of fluoride in animal feed following the EN 16279:2012 "Determination of fluoride content after hydrochloric acid treatment by ion-sensitive electrode method (ISE)".

In the frame of the Italian National Monitoring Plan for animal feed, 115 samples (raw material, complete and complementary feeding stuffs) were analysed in 2018 and 2019.

All samples were compliant to maximum limits. Fluorine was found < LOQ (40 mg Kg<sup>-1</sup>) in complete feed (n=42) while in complementary feed (n=41) 14% of samples contained fluorine in the range 34-143 mg Kg<sup>-1</sup> and 86% of samples were <LOQ. In raw materials (n=32) 60% of samples recorded concentrations between 935 – 1638 mg Kg<sup>-1</sup> (phosphate based) and 47 -150 mg Kg<sup>-1</sup> (meat, fish based).

[1] EFSA, 2004. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Fluorine as undesirable substance in animal feed.

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## 115 The Fitness for Purpose of Analytical Methods

Presenter: Helen Cantwell

Millions of measurements and tests are made every day in laboratories around the world. Method validation demonstrates that the results of these measurements and tests are fit for their intended use. While analysts are aware of the importance of method validation, exactly what needs to be done is not always clear. The Eurachem Guide The Fitness for Purpose of Analytical Methods gives practical advice on how to plan, carry out and report method validation.

Although the amount of validation required may vary, it is generally necessary to determine a number of performance characteristics such as working range, limit of quantification, precision and trueness. The Guide explains the different performance characteristics and suggests experiments that may be used to determine them. It also gives guidance on the evaluation of performance characteristics and advice on how to record and report validation studies.

The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics is available to download at [www.eurachem.org/index.php/publications/guides/mv](http://www.eurachem.org/index.php/publications/guides/mv).

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The Eurachem Method Validation Working Group aims to function as a centre of excellence in the area of method validation and is composed of members from countries throughout Europe.

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## 116 Eurachem Method Validation Working Group

Presenter: Helen Cantwell

The Eurachem Method Validation Working Group (MVWG) aims to function as a centre of expertise in the field by assembling and promoting best practice in method validation. The working group has members from countries throughout Europe and meet twice a year to provide guidance on method validation which will be applicable to all chemical analytical laboratories, meet the requirements for accreditation and address new developments within analytical chemistry.

The group organizes and contributes to international seminars and workshops on issues related to method validation within analytical chemistry. They ran an international workshop entitled Method Validation - Current Practices and Future Challenges in Ghent in 2016 and hosted a session on method validation in the 22nd International Mass Spectrometry Conference held in Florence in August, 2018.

The MVWG produce a guide to method validation entitled "The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics". In addition, the group have produced two supplementary guidance documents "Planning method validation studies" and "Blanks in method validation" and an information leaflet "The importance of method validation". These are available to download at [www.eurachem.org/index.php/publications](http://www.eurachem.org/index.php/publications).

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## 117 Occurrence of ergot alkaloids in cereals from Slovenia

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Ergot alkaloids (EA) are secondary metabolites produced mainly by fungi of *Claviceps* genus. They are mostly present in cereals and wild grasses. A maximum level of 1000 mg/kg of rye ergot (*Claviceps purpurea*) sclerotia has been established for feed containing unground cereals. However, EC recommended Member States to perform monitoring on the presence of ergot alkaloids in cereals intended for human consumption or animal feeding (Commission Recommendation 2012/154/EU). This study is the first reporting the ergot alkaloid presence in cereals in Slovenia in four consecutive years.

Wheat, barley, triticale, rye, oat and spelt were sampled in cooperation with farms and agricultural cooperatives in Slovenia from 2014 to 2017. Altogether, 517 cereal samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous determination of most important EA: ergometrine, ergosine, ergocornine, ergocryptine, ergotamine and ergocristine and corresponding -inine epimers.

In 2014–2017, EA were present in 87 out of 517 wheat samples (17 %). Incidence of positive samples were the highest in rye (54%), oat (50%) and spelt (30%), while in barley, wheat and triticale they were below 20%. The total alkaloid concentrations were in the range 14–4217 µg/kg and median concentration was 154 µg/kg. We observed the highest concentration levels of total EAs in grain samples with higher susceptibility to ergot infection (rye). The maximum concentration levels of total EAs were 4217 µg/kg and 4114 µg/kg in wheat and rye, followed by spelt (2682 µg/kg), triticale (2587 µg/kg) and oat (2191 µg/kg), while in barley the highest level was 1177 µg/kg.

In 2014–2017, each contaminated cereal sample contained one to twelve EA. More than two EAs were detected in 90% of all positive samples. The highest EAs co-occurrence in the positive sample represented group of 2–4 EAs (32%) and 5–8 EAs (36%), while one EA was present in 10% and more than 9 EAs in 20% of positive samples. The individual EAs, present in more than 10% of all and in more than 50% of positive samples were: ergometrine, ergosine, ergosinine, ergocristine and ergocristinine. Other EAs were present less frequently.

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## 118 Processing factors for pesticides in processed oilseeds for animal feed

E.W.J. Gerrits, M.J. Appel

Currently, limits for raw feed materials are used to assess processed feed ingredients. Processing factors can be used to make the raw feed ingredient limits suitable for processed ingredient assessment. When establishing processing factors, distribution of pesticides over different fractions due to processing must be taken into account. Knowledge and agreement on processing factors will help deciding whether subsequent risk assessment for processed feed ingredients is necessary.

The aim of the current study was to get indicative information on the distribution of various pesticides in the processed fractions of oilseeds. Therefore, 16-25 kg batches of three oil seeds (soybeans, rapeseed and sunflower seed) were spiked at 0.5 mg/kg with a mix of 11 pesticides within a range in log Kow from 0.57 to 6.6 (imidacloprid, carbendazim, metalaxyl, malathion, tebuconazole, pirimiphos-methyl, haloxyfop-P-methyl, chlorpyrifos-methyl, chlorpyrifos, deltamethrin and cypermethrin). The spiked oilseeds were pressed and extracted (rapeseed and sunflower seed) or only extracted (soybeans). Samples of untreated seeds, press cake, expeller, pressed oil and extracted oil were analysed using a validated LC-MS/MS method.

The European oil processing industry (Fediol) predicted the distribution of pesticides during oilseed crushing based on log Kow and fat content in the raw product. Fediol proposed processing factors in oil for pesticides with a log Kow > 3. Pesticides with a log Kow between 1 and 3 were expected to concentrate in oil for the largest share and therefore follow the processing factors calculation for pesticides with log Kow > 3. For pesticides with a log Kow < 1, no processing factors were proposed.

The preliminary results of the present research showed:

1. that the spiking procedure was accurate and repeatable on a semi-lab-scale for 16-25 kg;
2. a trend, similar to the Fediol calculation of processing factors, among the pesticides at log Kow > 3, but less similarity was found at log Kow < 3;
3. variance in pesticide distribution between the oilseeds.

These preliminary results should be confirmed on a pilot scale (100 kg batches) to mimic industrial processing, in particular focusing on pesticides with a log Kow 1-3 and pesticides with a negative log Kow.

### Author information

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## 119 Improvement of LC-MS/MS analysis of quaternary ammonium compounds by using a trap column

Marc Tienstra, Paul Zomer, Rita Boerrigter-Eenling, Hans Mol

Quaternary ammonium compounds (QACs) are dual-use substances and besides use as pesticides can also be applied as biocide or disinfectant. As disinfectants they are widely used in, for example, the cleaning of machinery used in the dairy industry and other food and feed producing factories. Their use in these type of installations means there is a chance of contamination of the products. Residual moisture rich products from agricultural-, food- and fermentation industry are used to produce liquid feed, typically prepared and used in pig- and cow-farms. Due to the high moisture content, this type of feed is sensitive for microbiological and fungal growth potentially causing animal health issues. Disinfection is applied at different stages in the liquid feed chain (i.e. production, transport, storage and distribution) causing possible contamination with residual disinfectants. Analysis of this type of matrix to check for residues of disinfection products is part of the Dutch National Feed monitoring program.

This work focuses on two groups of QACs: benzyldimethylammonium compounds with alkyl chains of C8-C18 (BAC C08-C18) and compounds like didecyldimethylammonium chloride with alkyl chain lengths of 8,10 and 12, colloquially named DDAC-C8-C12.

The EURL-SRM has published a method for the analysis of these compounds in different types of foods [1,2]. The parameters of this method show that the analysis of these compounds can be incorporated into a multi pesticide LC-MS/MS method. During development of a method for the analysis of products of animal origin we found that some of the BAC/DDAC compounds are present in the background of all analyses. Tests showed they were not originating from the solutions used to clean the lab glassware or the soap used in the laboratory. It was found out that the main contributor to the background contamination was the LC-system used. This required a special solution to solve this issue.

In the analysis of certain industrial pollutants (perfluorinated compounds such as PFAS) the same background contamination problem arises. In the PFAS analysis it is customary to use a trap column, placed between pump outlet and autosampler [3], to trap and delay the chromatography of the compounds in the background and separate them chromatographically from the compounds present in the sample. This approach was tested and also here demonstrated to solve this issue: a substantial decrease in background levels and no impact (only small increases in retention times) on the analysis of other pesticides included in the same method. The instrumental set-up and the validation results for the BAC and DDAC compounds in liquid feed for piglets will be presented.

## 119 Improvement of LC-MS/MS analysis of quaternary ammonium compounds by using a trap column

Continued

[1] EURL-SRM, Analysis of Quaternary Ammonium Compounds (QACs) in Fruits and Vegetables using QuEChERS and LC-MS/MS Version 5 (last update: 24.03.2016) [https://www.eurlpesticides.eu/userfiles/file/EurlSRM/EurlSRM\\_meth\\_QAC\\_ShortMethod.pdf](https://www.eurlpesticides.eu/userfiles/file/EurlSRM/EurlSRM_meth_QAC_ShortMethod.pdf)

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## 120 The EUginus GMO database

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Maintaining feed safety is a fundamental challenge in modern agriculture. The feed industry as well as competent authorities need to continuously monitor feed materials in order to avoid the production and use of feeds containing undesirable components. In the EU specific legal limits are established and methods are developed to detect various unwanted components. However, globalisation of the feed business complicates a consequent monitoring due to the diverse legal regulations all over the world. Especially the monitoring of genetically modified organisms (GMOs) poses a challenge since both the definition of a GMO and the authorisation of certain GMOs differ around the world. For example, novel genome editing organisms such as those developed using CRISPR-Cas, are categorized as GMOs under EU legislation but are not regulated/deregulated in other countries.

Therefore, the European market needs efficient tools to assist in identifying GMOs. Centralised information on existing GMOs (authorised and non-authorised, conventional or genome edited), available detection and identification methods as well as up-to-date authorisation status should facilitate consistent monitoring in the EU.

Here we present the EUginus GMO database ([www.euginus.eu](http://www.euginus.eu)), an initiative of Wageningen Food Safety Research (WFSR), Wageningen UR (Wageningen, NL) and BVL - the Federal Office of Consumer Protection and Food Safety (Berlin, DE). This database supports competent authorities and private users, who seek comprehensive and accurate information on GMOs. EUginus provides four major interconnected modules: (1) The GMO module lists existing conventional GMOs and genome edited organisms and enables sorting by specific criteria like trait, company or genetic elements. It also provides detailed information on the molecular characterisation including annotated sequences. (2) The detection module contains detection methods including reference materials and tools supporting the development of screening strategies. (3) The analysis module provides a tool for the interpretation of screening test results. (4) The authorisation module offers detailed authorisation status and EU application details on food and feed. Additionally, we designed and integrated in EUginus a novel GMO genetic element thesaurus (GMO-GET) that enables the communication with and harmonisation of comparable databases like the BCH registry.

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## 121 Determination of urea in animal feed with liquid chromatography-mass spectrometry (LC-MS)

Robin S. Wegh, Ron Berentsen, Benedikt Brand\*, Tina Zuidema, Jacob de Jong

Urea is a feed additive that is used as a source of nitrogen for the production of protein by rumen microbes through the production of ammonia and carbon dioxide. The use of urea as a feed additive is only allowed for ruminants with a functional rumen with a maximum content of 8800 mg/kg complete animal feed. When too much ammonia reaches the liver, the liver can no longer process it and toxic effects and even death can occur. With these rules, it is important to be able to check the real urea content in an animal feed. The aim was to develop a robust method for the analysis of urea in animal feed with a high specificity. In order to effectively monitor urea concentration in animal feed. Two reasons for monitoring are, label control and illegal pretense of urea in animal feed.

In 2018 a method was developed and fully validated to determine the concentration of urea in animal feed using ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) with atmospheric-pressure chemical ionization (APCI).

Application of the method in practice and result comparison between the LC-MS method and official EU method will be presented.

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## 122 Inventory of relevant biocides for animal feed safety - Approval status and overlap with other legislation

Nathan Meijer, Robin Wegh, Hans Mol, Jacob de Jong

Residues of biocidal substances may remain in the feed after indirect application to storage or transport containers. The presence of these residues raises concerns on the safety of the feed materials. The primary objective of this study was to assess which biocide residues that may be present in feed are most relevant for monitoring purposes. The group of biocides encompasses many substances with a variety of different uses. In the EU, the legal framework is set in Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. An analysis was made on the overlap between the lists of biocides relevant for the 'food and feed area' (product type (PT) 4), and drinking water (PT-05); and between these and legislation on pesticides, undesirable substances in feed, and feed additives. Several active biocidal substances were identified as being of potential interest for monitoring purposes due to overlap or discrepancies with other legislation or approval statuses. Discrepancies or overlap between the approval status of substances depending on its use provides the possibility of incorrect or fraudulent use. Therefore, vigilance in monitoring is advised. Residues in feed materials may be present because of different uses; from biocides, pesticides, and/or feed additives. The legal limits for each of these uses may differ, but an assessment thereof was beyond the scope of this study.

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## 123 Trace Elements and Heavy Metals Analysis in Animal Feed by ICP-MS and ICP-OES

Veronica Gubarkova, Helen Cantwell and Niamh Fitzgerald

The principal responsibility of the Animal Feedingstuffs Section in The State Laboratory is in providing a comprehensive analytical and advisory service to Government Departments and Offices thereby enabling them to implement and formulate the technical aspects of National and EU Legislation. Our main client is the Department of Agriculture, Food and the Marine (DAFM) – from whom we receive approx. 1,500 samples per year.

We carry out analysis on different feed material types: compound feedingstuffs, feedmaterials and premixtures. This poster will focus on the work carried out on the organic and inorganic samples analysed for trace elements and heavy metals, including inorganic arsenic analysis as well as our newly developed single particle ICP-MS method for gold and silver nanoparticles. Methods used for official control are validated and accredited in accordance with ISO 17025.

The State Laboratory is designated as a NRL for heavy metals and feed additives in animal feed. There is an emphasis on food safety and animal welfare in the Trace Element area of analysis. Diets for farm animals usually contain a mineral/trace element/vitamin supplement, and diets lacking in essential mineral elements cause deficiency symptoms in animals. These symptoms can be eradicated or prevented by adding extra supplies of some minerals to the diet. For each sample received, the trace element content of feedingstuffs must be declared on the label and analysis is carried out to establish compliance with the declared content. The focus is on achieving fast turnaround times for compliant samples.

Heavy metals are considered undesirable substances which present a potential danger to animal or human health. The presence of undesirable substances in feed is controlled by European Parliament and Council Directive 2002/32/EC which sets maximum permitted levels (MPLs) for these substances. Feedingstuffs tested in our laboratory with levels of undesirable substances greater than the MPLs are deemed to be unsafe by our client (DAFM) and will be withdrawn and disposed of outside the feed and food chains.

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## 124 Workflow for the identification of 'unknown' substances in animal feed

Robin Wegh, Milou van de Schans, Rosalie Nijssen, Bjorn Berendsen

Regularly biological effects or instrumental signals are observed in animal feed samples that cannot be explained by the presence of a chemical substance that is included in target mass spectrometric methods. To identify the compound responsible for the biological effect, a workflow is developed based on multi-disciplinary collaborations. This includes analytical chemistry (mass spectrometry, NMR), biochemistry (biosensors, DNA analysis), physical chemistry (microscopy) and knowledge of bioactive compounds.

The starting point of the search for 'unknowns' can be threefold: (1) a casus from the field, for example negative health effects, (2) an observed effect in a bioassay and (3) a suspect signal in a chemical analysis, e.g. high resolution mass spectrometry. The developed workflow was assessed using two artificial cases. In both cases, the identity of the 'unknown' was successfully confirmed allowing application for a real feed sample and supplement. We experienced that the success rate of identification of the unknown compound increases when multidisciplinary research is applied in which biochemistry, analytical chemistry and physical chemistry is combined. The applied workflow, success factors and examples will be presented.

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## 125 Transfer of poly- and perfluoroalkyl substances from feed to hen tissue and eggs

Janine Kowalczyk<sup>1</sup>, Bernd Göckener<sup>2</sup>, Maria Eichhorn<sup>2</sup>, Mark Bücking<sup>2,3</sup>, Helmut Schafft<sup>1</sup>, Monika Lahrssen-Wiederholt<sup>1</sup>, Jorge Numata<sup>1</sup>

A feeding study with laying hens (Lohmann brown) was conducted to quantify the feed to hen tissue and egg transfer of perfluoroalkyl acids (PFAA) and related precursors, which belong to the class of poly- and perfluoroalkyl substances (PFAS) – a group of more than 4700 compounds. The experiment was performed with 12 hens fed by a controlled PFAS contaminated diet for 25 days (exposure period) followed by a 42 days depuration period with uncontaminated feed. During the study, feed intake was recorded daily and eggs were collected every second day, then separated into yolk and albumen and pooled for PFAS analysis. After slaughter, PFAS-analysis was also performed for serum, muscle tissue, kidneys and liver.

PFAS target analysis was performed using HPLC-MS/MS, whereas the Total Oxidizable Precursor (TOP) method was additionally applied to quantify the precursors in feed and eggs. The analysis revealed substantial amounts of the precursors N-methyl and N-ethyl perfluorooctane sulfonamidoacetic acid, perfluorooctane sulfonamidoacetic acid (FOSAA) and perfluorooctane sulfonamide (FOSA). Up to 8 PFAAs of different chain lengths (C4-C8) were likewise detected in feed, animal tissues, and yolk, with only minor amounts in albumen.

One-compartment toxicokinetic models were fitted to the experimental data for each substance. A model including precursors to perfluorooctane sulfonic acid (PFOS) biotransformation was fitted on a molar basis. In tissues and eggs, the highest bioaccumulation was found for perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid (PFHpS), PFOS, and perfluorooctanoic acid (PFOA). Even after the depuration period, low levels of PFHxS (all samples), PFOS, and FOSAA (yolk) were still measurable. The egg elimination half-lives of PFOS and aforementioned precursors were estimated to be 4.3 days, while the transfer rates of PFOS and all precursors taken together were 0.99. The transfer rate of PFOA was around 0.49. PFHxS and PFHpS showed apparent transfer rates of >100%, which is hypothesized to indicate the presence of further unknown precursors.

Overall, the study highlights the significance of PFAA precursors to total PFAS burden of hens and subsequent transfer into food of animal origin. The toxicokinetic model was derived as a prediction tool for PFAS in the feed-egg-chain.

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## 128 LC-MS Method for Rapid Simultaneous Determination of BHA and BHT

Jennifer Gjerde, Annbjørg Bøkevoll, Robin Ørnstrud

Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) are synthetic phenolic antioxidants widely used as feed additive in fish feed. Synthetic antioxidants have also been used in pharmaceutical, cosmetic and food industries. BHA and BHT are considered generally safe in approved concentrations. However, BHA and BHT have shown carcinogenic effect in animals. The wide use of BHA and BHT subject these substances for further research. Thus, questions concerning exposure, bioaccumulation, and toxicity of BHA and BHT should be further explored.

The levels of BHA and BHT are monitored by the Norwegian Food Safety Authority in a feed monitoring program. Several laboratories measure BHA using HPLC-UV method and BHT using HPLC-fluorescence method. Incorrect results caused by the presence of interfering substances using these methods have been observed. Thus, the need of sensitive and selective analytical method and expertise in examining and interpreting laboratory results are of importance. In this study, we developed a method that simultaneously determine BHA and BHT. A method using high resolution LC-MS was developed to determine and quantitate BHA and BHT and its degradation products in fish feed.

### Author information

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Field: Mass Spectrometry, chromatography, method development, pharmacokinetic, pharmacogenetics and clinical research.

## 129 Determination of urea in compound feed for ruminants: Validation of the spectrophotometric method in Commission Regulation (EC) No 152/2009

Ursula Vincent, Jörg Stroka, Carlos Oliveira Gonçalves, Katrien Bouten, Hakan Emteborg, Christoph von Holst

Urea is a feed additive authorised in ruminant feed by Commission Implementing Regulation (EU) No 839/2012 with a maximum content in compound feed of 8800 mg kg<sup>-1</sup>. Commission Regulation (EC) No 152/2009 displays the official method for the determination of urea in feed, but does not specify the type of feed for which the method is applicable. Following reports from some Member States stating that the official spectrophotometric method is not fit for purpose for other feed than ruminant feed and given the fact that no method performance characteristics are set, the European Union Reference Laboratory for Feed Additives Control (EURL-FA Control) organised an interlaboratory exercise for the validation of this method. The collaborative study was carried out on 6 different test materials prepared from sheep and cattle compound feeds containing urea at various mass fractions. Nineteen laboratories enrolled for the study and eighteen laboratories from ten EU Member States submitted results.

The application of the spectrophotometric method foresees the measurement at two wavelengths, namely at 435 nm and 420 nm. The obtained relative standard deviation for repeatability (RSDr) of the measurements at 435 nm ranged from 1 % to 14 % depending on the matrix/mass fraction combination. The corresponding values for relative standard deviation for reproducibility (RSDR) at the same wavelength, varied between 7 % and 18 %. At 420 nm, the RSDR was in all cases, except in sheep feed at 3000 mg kg<sup>-1</sup>, less than 20 %. This set of results confirmed that the official spectrophotometric method described in Commission Regulation (EC) No 152/2009 is fit for the purpose of the determination of urea in ruminant feed at target level.

The general outcome of the study led to the conclusion that the Commission Regulation (EC) No 152/2009 should be revised as regards the official spectrophotometric method with a restriction of the scope to ruminant compound feed and the inclusion of the method performance characteristics obtained from the present interlaboratory exercise in this Regulation.

### Author information

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## 130 Italian monitoring plan on carry-over occurrence of antibiotics in feed

Rosa Avolio, Sabina Pederiva, Sara Morello, Maria Ines Crescio, Giuseppe Ru, Fabrizio Grifoni, Maria Cesarina Abete, Daniela Marchis

Antibiotics residues in feed can have an impact on animal health, as they may contribute to the antimicrobial resistance (AMR) occurrence. During feed production process, carry-over may happen, as an unintentional but unavoidable presence of traces of active substances in a feed batch to the next ones. So far, the European Union (EU) rules do not provide any carry-over action limit for antibiotics in feed, while since 2015 the Italian Competent Authority established action limits based on the 'as low as reasonably achievable' (ALARA) principle.

This study aimed to investigate antibiotics at carry-over level in feed, as part of the Official Feeding-stuffs Controls carried out between 2017 and 2020 in the North-West of Italy (Piedmont, Liguria and Aosta Valley). Overall, 785 feed samples were collected and processed, of which 61% complete feed, 18% complementary feed, 15% farm feed and 6% raw materials. Analyses were carried out applying accredited in-house methods performed by high-performance liquid chromatography with diode-array detector (HPLC-DAD), HPLC with fluorescence detector (HPLC-FLD) and HPLC with electrospray tandem mass spectrometry (HPLC-MS/MS), for the determination of different classes of antibiotics.

Collected data showed a decreasing trend of non-compliance samples along the examined period. The most identified antibiotics were tetracyclines, which appeared to be the most used antimicrobics in feed, due to their cost-effective activity compared to other molecules. Furthermore, pigs were confirmed to be the most treated species.

Many Countries have banned antibiotic use as growth promoters, nevertheless it has been demonstrated that antibiotics are often used prophylactically, particularly in intensive pig farming to prevent production loss.

The decrease of positive samples collected over the years suggests that existing monitoring plans are effective to control the carry-over occurrence and to contain the development of AMR. Besides, the application of quality management schemes, more awareness of feed business operators and improved animal welfare conditions appear to contribute positively to a better application of good manufacturing practices (GMPs).

The results highlighted the need to establish relevant requirements for detection of carry-over and data collection could be useful to set better evaluated action limits of drug residues in feed.

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### 131 Aluminium occurrence of feed from northwestern Italy

Stefano Falsetti<sup>1</sup>, Alessandra Griglione<sup>1</sup>, Paola Brizio<sup>1</sup>, Maria Cesarina Abete<sup>1</sup>, Antonio Curcio<sup>2</sup>, Stefania Squadrone<sup>1</sup>

The EFSA Opinion regarding the Safety of aluminum (Al) from dietary intake [1] demonstrated that the main route of exposure to Al for humans and animals is through food. In fact, most unprocessed foods typically have less than 5mg kg<sup>-1</sup> of Al. The survey indicated cereals and vegetables as important contributors (>10%) to dietary exposure to Al.

**OBJECTIVE:** Since no data are still available about Al in Italian feed and this element is not part of official and/or regional control programs, the National Reference Centre for the Surveillance and Monitoring of Animal Feed (CReAA) performed a monitoring in Piedmont to evaluate the occurrence of Al in feed materials.

**METHODS:** Feed samples of rice, wheat, corn, barley, triticale, oats, and soybeans were collected (n=71). After homogenization, 1.5g of sample was added with 7mL of HNO<sub>3</sub> (70% v/v) and 1.5mL of H<sub>2</sub>O<sub>2</sub> (30% v/v) and subjected to mineralization in a microwave digestion lab station (ETHOS 1, Milestone S.r.l, Sorisole, BG). Al quantification was carried out using an ICP-MS (Thermo), tuned daily before each analytical trial. The limit of quantification (LOQ) is 0.010mg kg<sup>-1</sup>.

**RESULTS AND DISCUSSION:** In the study area (Biella province, Piedmont) higher Al concentrations were found than those reported for similar matrices in other countries [2], in particularly for rice (mean value 30mg kg<sup>-1</sup>) and soy (89mg kg<sup>-1</sup>), two plant species widely used in human and animal nutrition. This finding is related to soil characteristic and to the physiological characteristics of the plant species. Al is the third most abundant element in the lithosphere, mainly found as Al-silicate in feldspar, micas, and clays. The soil of the studied area is particularly enriched of hydrated aluminosilicates. Rice is one of the most Al-tolerant cereal species, while soy as leguminous plants is colonized by mycorrhizae, symbionts fungi that absorb nutritional macro and microelements, transfer them to plant receiving elaborated lymph in exchange. However, the improving of the absorption of soil nutrients also corresponds to a greater capacity of mobilize and accumulate metals from soil.

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### 132 A first study of rare earth elements (REEs) in organic production for feed

Stefania Squadrone<sup>1</sup>, Stefano Falsetti<sup>1</sup>, Alessandra Griglione<sup>1</sup>, Andrea Orrù<sup>2</sup>, Maria Cesarina Abete<sup>1</sup>

Despite their name, lanthanides or rare earth elements (REEs) are a group of chemical elements that represents the fifteenth most abundant component of the earth's crust. According to their atomic number, they are subdivided into light REE (LREE), including lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd) and samarium (Sm); and heavy REE (HREE), including gadolinium (Gd), europium (Eu), terbium (Tb), dysprosium (Dy), thulium (Tm), ytterbium (Yb), holmium (Ho), erbium (Er), lutetium (Lu), scandium (Sc) and yttrium (Y) [1]. In the last 20 years, REEs have been widely utilized, especially in China, as fertilizers and in animal husbandry as growth promoters. The main sources of lanthanides in nature are bastnasite, monazite, and laterite clays; however, the occurrence of lanthanides in the environment is also related to industrialization processes since they are utilized in high technology products due to their excellent physicochemical properties. Previous investigation performed in feed and raw materials from northwestern Italy demonstrated the occurrence of lanthanides (SREEs) in the range 2700-610 mg kg<sup>-1</sup> [2]. The detection of traces of Lanthanides in feed may be due to the use, during primary agricultural production, of REE-based artificial fertilizers, especially phosphates. The use of these products is prohibited in organic farming but the use of Thomas slags, a by-product of steel production, containing calcium, phosphorus and small amounts of other elements, is allowed.

**OBJECTIVE:** Since no data are still available about these emerging contaminants in organic productions, the National Reference Centre for the Surveillance and Monitoring of Animal Feed (CReAA) and the National Reference Center for Organic Production (CRNPB) aimed to detect REEs in organic production utilized for animal feed.

**METHODS:** Feed samples (n=20) were homogenized, then 1.5 g of sample was added with 7 mL of HNO<sub>3</sub> (70% v / v) and 1.5 mL of H<sub>2</sub>O<sub>2</sub> (30% v/v) and subjected to mineralization in a microwave digestion lab station (ETHOS 1, Milestone S.r.l, Sorisole, BG), equipped with a 10 positions rotor for high pressures polytetrafluoroethylene (PTFE) digestion tubes. Lanthanides quantification was carried out using an ICP-MS (Agilent 1260 Infinity II). Instrument was tuned daily before each analytical trial. The limit of quantification (LOQ) is 1.0 mg kg<sup>-1</sup>.

**RESULTS AND DISCUSSION:** REEs are nonessential elements for life, however they have been scarcely investigated and remain unregulated since are not part of monitoring environmental/ safety plans.

## 132 A first study of rare earth elements (REEs) in organic production for feed

Continued

In the analyzed samples SREEs were found with the following decreasing trend: 240 mg kg<sup>-1</sup> in complementary feed, 60 mg kg<sup>-1</sup> in complete feed and 39 mg kg<sup>-1</sup> in raw materials (mean 71 mg kg<sup>-1</sup>). Results have shown that REEs concentration in organic production are an order of magnitude lower than in previous finding in feed [2]. The most represented element was Y (mean 41 mg Kg<sup>-1</sup>) followed by La, Ce and Sc (respectively 14, 9.8 and 9.6 mg Kg<sup>-1</sup>).

REEs have lithophilic characteristic and terrestrial vegetation reflect the soil composition of the location in which they grow. In addition, the transfer of metals from soil to plant is a possible route of contamination for the food chain, then the occurrence of REEs should be carefully considered. The concentrations registered in the organic materials for feed formulation were low and did not constitute concern from animal health.

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## 133 Re-authorisation of gelatine and collagen from ruminant origin in non-ruminant feed: a new analytical challenge for the control of the feed ban?

O. Fumiere<sup>1</sup>, M.-C. Lecrenier<sup>1</sup>, J. Zagon<sup>2</sup>

In 2020, an EFSA scientific opinion gave green light to the use of ruminant collagen and gelatine in feed for non-ruminant farmed animals. Based on this text, the EU Commission proposed a revision of the feed ban provisions authorising the use of these ingredients. This lifting would allow the use of "former foodstuffs" made of bakery products, sweets, yogurt, desserts, ice cream,... containing collagen hydrolysates and gelatine rather than the use of ruminant collagen and gelatine as such in feed. CRA W and BfR initiated a study on the possible implications of such a re-introduction on the capacity to detect processed animal proteins (PAP) from ruminant origin in feed. Collagen hydrolysates, gelatines and "former foodstuffs" were collected with the support of the industry. The study aimed to investigate, if ruminant DNA is detectable in the samples using the official DNA extraction- and PCR method. On the contrary, gelatine and collagen being reputed to be powerful PCR inhibitors (Liu et al, 2015; Schrader et al., 2012), it was relevant to assess the possibility that traces of ruminant PAP in co-presence of collagenous material might not be detected using the same PCR protocol. In addition, the same samples were also submitted to a mass spectrometry (MS) protocol targeting ruminant proteins, including collagen, to distinguish between ruminant by-products (unauthorized or authorized).

Clearly, amplifiable DNA is extractable in the samples analysed. DNA amounts are highly influenced by the processing but the remaining DNA is detectable in a majority of the samples. PCR signals are low to moderate with collagen and gelatine samples. However, strong PCR signals are observed with "former foodstuffs". These results let assume that in certain cases low traces of "former foodstuffs" in a feed may yield a ruminant PCR response giving rise to "false" suspicions of prohibited materials. Moreover, the absence of PCR inhibition is demonstrated using two different strategies. The PCR inhibition properties of collagen and gelatine are most probably dependent on the DNA extraction and purification protocol and the official protocol is efficient to discard the PCR inhibitor molecules from collagen and gelatine. MS analyses performed on the same set of samples show that collagen biomarkers are detected in all samples containing bovine/ruminant gelatine. As for PCR, signal intensity varies depending of the collagen source (hide or bone) and the gelatine processing. The use of milk biomarkers also shows that the majority of the PCR signal is probably due to the use of milk in former foodstuffs.

These results show the complementarity of PCR and MS approaches in the context of the use of former foodstuffs in animal feed. Nevertheless, in case of a simultaneous presence of PAP and former foodstuffs in a feed, the origin of the PAP would remain most probably unknown.

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### 134 Performance of different handheld NIR spectrometers to evaluate grass silage quality

Ph. Vermeulen, J.A. Fernández Pierna, N. Chamberland, V. Decruyenaere, E. Froidmont, O. Minet, B. Lecler, V. Baeten

This study aims to develop analytical solutions for predicting quality parameters of forages based on the use of handheld spectrometers. This kind of spectrometers have been in full expansion for already few years and allow predicting directly on site the quality of forages, enabling then a better knowledge of the forage resources produced at the farm level.

The goal of this study is to compare the performance of three recent developed spectroscopic handheld devices, namely the Viavi Micronir 1700 (908-1676 nm), the OceanOptic Flame-NIR (936-1665 nm) and the ASD FieldSpec (350-2500 nm) to predict fresh and unground grass silage in the framework of a smart dairy cows feeding. Moreover, a benchtop spectrometer, namely the FOSS XDS, is also used for comparison, as being a historical reference for such kind of analysis.

For this study, 181 samples of grass silage were collected between 2018 and 2020 at 60 different silage silo in Wallonia (Belgium). All samples were measured with the three handheld devices in fresh and unground. Moreover, an alternative set of 19 validation samples was also obtained from the same period and coming from different locations as in the calibration set. For all this dataset, reference values were obtained by prediction with a FOSS DS2500 on dried and ground samples for protein, total ash, crude fiber, acid and neutral detergent fiber constituents. Dry matter was obtained by drying samples at 60°C during 48 hours.

Chemometric tools comprised the building of calibration models using the classical Partial Least Squares (PLS) regression as well as the use of a local based PLS algorithm.

This study highlighted the fact that the dry matter is relatively well predicted with all the devices using both PLS and the local based PLS method. However, low performances were obtained for models built for the other chemical parameters. This could be maybe due to the fact that the validation set used does not cover the large variation present in the calibration set for these parameters.

This study was realised in the framework of the Effort project funded by the CRA-W. More information on <https://www.cra.wallonie.be/en/effort-2>.

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### 135 Proof of storage stability of FUMzyme® in swine and poultry feed

Silvia Labudova\*, Doris Hartinger, Karin Schoendorfer, Barbara Doupovec, Oliver Greitbauer, Dian Schatzmayr

Fumonisin (FUM) are secondary metabolites produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisin B1 (FB1) is considered as the most prevalent and toxic one.

Diverse technologies have been suggested for reducing FUM contamination. An efficient method of reduction should be able to remove or inactivate the mycotoxin without producing toxic residues and thus prevent the impact of FUM on livestock productivity.

Biomin® developed an enzyme fumonisin esterase FumD (FUMzyme®), authorized in the European Union as a feed additive that acts by hydrolysis of the two tricarballic acid (TCA) side chains of FUM, resulting in the formation of the non-toxic hydrolysed FUM. The effectivity of FUMzyme® was confirmed in various animal species using the Sa/So ratio as a biomarker. Clear evidence was shown that FUMzyme® is able to facilitate gastrointestinal degradation of dietary FB1 in pigs, turkeys, chickens and ruminants and to prevent an increase of the Sa/So ratio in serum.

Besides the proof of efficacy, authorization of a feed additive also requires providing evidence on how the quality of a substance varies with time under the influence of a variety of storage conditions. In this study, conducted for regulatory purposes, the storage stability of FUMzyme® was tested in two types of feed representing a standard swine feed (feed phase III) and a poultry mash feed (broiler starter). Three independent batches per feed type were investigated. Tested batches were stored at room temperature (22 ±2 °C) without humidity control in the commercially used paper bags over 6 months. Sampling was done on day 0 as well as after 2, 4 and 6 months of storage. We tested the enzymatic activity by a validated method measuring TCA units/kg feed using LC-MS/MS.

Results confirmed no significant influence of time on the activity of FUMzyme® in both tested feed types. Recovery of TCA activity in swine feed and poultry feed reached 94, 94 and 95 % and 91, 99 and 102 %, respectively. In conclusion, the investigation confirmed the stability of FUMzyme® in swine and poultry feed over at least 6 months of storage.

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### 136 Method of assessing homogeneity of compound feed in the national feed supervision

Slawomir Walczynski, Waldemar Korol

The aim of the work was to present the method of assessing the homogeneity of compound feed for the purposes of official control. An appropriate instruction was approved by the Chief Veterinary Officer. The study was based on data collected during many years of proficiency testing (PT) in the field of homogeneity of compound feed, including assumptions based on the Nordtest TR 537, Regulation 691/2013 and ISO 6498. The evaluation process consists in collecting 6 incremental samples, each 100 g in weight, from a controlled batch of the product. Average values of  $x_A$ , the content of the selected component from a series of six incremental samples performed in duplicate are calculated using all results,  $n = 12$ . Similarly, the standard deviation SD is calculated for  $n = 12$ . Accepted value of the degree of mixing, the measure of which is the coefficient of variation  $CV_m$  of the results of testing the content of the selected component, should not exceed 10%. In the case of  $CV_m > 10\%$  the coefficient of technical variation ( $CV_h$ ) (%) is calculated, describing the homogeneity of the method not charged with analytical variability, which then determines the degree of mixing. According to the Instructions, proficiency testing was carried out with the participation of official supervision laboratories. Two poultry feed mixtures were used as the test material. The participants measured the content of macronutrients (calcium, chloride) and micronutrients (zinc, copper) in two serial samples. In PT, 98% of satisfactory results were recorded, and 1% of questionable and unsatisfactory results each. All the submitted results of the mixtures analyzes, on the basis of which the laboratories calculated the  $CV_m$  coefficient, confirmed the appropriate homogeneity of the mixtures used in the study. No  $CV_m$  result exceeded 10% and there was no need to calculate  $CV_h$ . The average values assigned to the elements tested were: chlorides - 4.1%, calcium - 2.9%, zinc - 3.5%, copper - 3.4%. The obtained data from PT confirmed the correctness of the adopted assumptions of the Instruction, at the same time confirming the competences of the participants in the assessment of homogeneity of compound feed.

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### 137 Selected contaminants in commercially produced fish feed and feed ingredients in Norway from 2006 to 2020

Veronika Sele, Monica Sanden, Rita Hannisdal, Anne-Katrine Lundebye, Marc Berntssen & Robin Ørnsrud

Norway is a large producer of fish feeds for Atlantic salmon (*Salmo salar*), with a production of 2.0 million tones fish feed in 2020. Traditionally, fish feed was composed of marine raw materials, e.g. fish meal and fish oil. However, since the 1990s the composition of fish feed has changed from being marine based to having a higher inclusion of plant materials. In 2018 insects were approved as a feed ingredient for fish feed, thereby introducing a new raw material. The change in raw material used for fish feed may affect both the nutrient profile and contaminant levels both in the feed and in the salmon tissue.

Annually, several contaminants are monitored in commercially produced fish feed and feed ingredients in Norway. The aim is to monitor potential risks related to the production of fish feed and to assure that the levels of contaminants are below the legislative limits. In this study, selected contaminants; dioxins, PCBs, mercury and arsenic in complete fish feed retrieved through the Norwegian monitoring program were evaluated in terms of time trends, for the years 2006 to 2020. Furthermore, the levels of these contaminants in various feed ingredients, including fish and plant meal, fish and plant oil, insect meal and premixes were evaluated as potential sources of these undesirable substances in complete feed.

The data show that the median level for dioxins, dl-PCBs, PCB6, mercury and arsenic in the feeds for these years ranged between 0.31-0.86 ngTEQ/kg, 0.21-1.1 ngTEQ/kg, 3.3-13 µg/kg, 0.01-0.07 mg/kg, 1.8-6.8 mg/kg, respectively. Between 2006 and 2020, the levels in the feeds initially declined rapidly, then more gradually. Differences were seen in the levels of these contaminants in the feed ingredients, with marine feed ingredients having higher concentrations than plant-based feed ingredients. The results show that the feed ingredients can contribute with different levels of contaminants to the complete feed, and the change in levels of contaminants in complete feed over time is likely a reflection of the change in raw materials used in fish feeds.

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## 138 Austrian Grain Harvest Survey 2020

Sabine Masching, Michael Schibich, Bernhard Bürgmayr

**Objective:** 95% of mycotoxins, secondary metabolites of fungi, are already formed in the field and harvested together with the respective grains. This group includes most of the *Fusarium* toxins such as deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FUM). In addition, inadequate storage conditions (humidity, temperature) can promote additional fungal growth when the grains are stored, which results in further formation of so-called storage mycotoxins (e.g. aflatoxins, ochratoxins). Even moderate mycotoxin concentrations in the feed can have a negative impact on the health and performance of farm animals. The objective of this review is to assess mycotoxin occurrence in Austrian grains in 2020.

**Methods:** Results of numerous studies indicating the high and frequent occurrence of mycotoxins in grains, and the importance of monitoring them as a part of mycotoxin risk management program. The BIOMIN® Mycotoxin Survey, which runs for 15 years, is a valuable tool for the risk assessment of mycotoxins in feed. Almost 250 samples (barley, wheat, triticale, oats and maize) originating mainly from Burgenland, Lower Austria, Upper Austria, Styria as well as Carinthia were analyzed by means of ELISA for DON, ZEN and FUM in the course of the Austrian grain harvest survey 2020.

**Results and discussion:** The results (Table 1) show that wheat, triticale and maize pose a high risk with regards to DON. The ZEN contamination is in the medium risk range and FUM showing a rather moderate risk level. A sample of organic oats was also analyzed, highly contaminated with 1963 ppb of DON.

Table 1. Mean mycotoxin contamination (ppb) of Austrian grain samples, harvest 2020

	Barley	Wheat	Triticale	Maize
Number of samples	86	46	10	104
Barley	394	1321	1065	1180
Barley	43	76	63	165
Barley	n.a.	n.a.	n.a.	686

n.a. samples were not analysed for FUM

Very important here is the fact that the majority of the examined feed samples (> 90%) are contaminated with more than one mycotoxin. The combination of several toxins causes an increased health risk even in the moderate contamination range (synergistic effects). These results clearly show that mycotoxins pose a threat in Austrian farms. It is therefore always advisable to carry out preventive mycotoxin risk management in order to ensure the health and performance of the animals.

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## 139 Analysis of illicit drugs to discover distribution to crops and food producing animals

Robin S. Wegh, Johan Lasaroms, Gerrit Bor, Tina Zuidema, Anthony Verschoor

The production of illicit drugs (for example MDMA, Amphetamine and Methamphetamine) and the number of illegal laboratories producing these drugs and storage locations is rapidly increasing. In the Netherlands up to one hundred of these laboratories are discovered on a yearly base and besides that between two hundred and three hundred, illegal dump spots with waste belonging to the production of illicit drugs are discovered. The most common dump spots are in nature reserves or parking places but also rest products are discovered in a manure sink of a farm. When the contaminated manure is spread over agricultural land, there will be a risk of transfer to crops and food producing animals. To determine whether this contaminated manure poses a risk, the stability of the illicit drugs and marker components in different environmental samples (manure, soil and water) was tested. A quantitative LC-MS and a GC-MS method was developed to analyse MDMA, Amphetamine, Methamphetamine and its precursors in different agricultural products.

The applicability of the method used for environmental and agricultural samples will be presented.

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## 140 Screening and identification methods for official control of antibacterial substances in animal feed

Monika Przeniosło-Siwczyńska, Ewelina Patyra, Aleksandra Grelik, Maja Chyłek-Purchała, Beata Kozak, Krzysztof Kwiatek

Animal feeding has a clear impact on food safety, and animal feeds must meet the requirements regarding contaminants and antibacterial agents. Monitoring of antibiotics in feed is important from viewpoint of controlling contaminants within the food-processing chain, potentially causing deleterious health effects in consumers and animals, and violating good manufacturing guidelines.

Available methods of analysis have to be suited for the effective and efficient control of the possible illegal use of banned veterinary antibiotics in feed to ensure feed and food safety. The methods should be implemented in an overall control strategy, ranging from fast and cheap screening methods to confirmatory methods, applying sophisticated instrumentation. Microbiological plate tests may be regarded as screening methods, whereas liquid chromatography with fluorescence detection and especially liquid chromatography coupled with mass are powerful techniques for confirmatory analysis. In the study screening was done with the microbiological 8-plate test which allowed to discriminate between different groups of antibacterial compounds. Plates were designed for the detection of antibacterial substances owing to its pH and a sensitive bacterial strain. *Bacillus subtilis* BGA, *Bacillus cereus* ATCC 11788, *Kocuria rhizophila* ATCC 9341 and *Escherichia coli* ATCC 11303 were chosen as the sensitive strains for analysed substances. To obtain qualitative and quantitative confirmation of the presence of the antibacterial substances the chemical procedures were designed based on liquid chromatography with fluorescence detection and mass spectrometry. During 2013-2017, a total of 214 samples of feedingstuffs were analysed according to a 2-step control strategy. The samples were taken as part of the national feed monitoring plan. Our study showed that out of the 173 samples tested, contaminating antimicrobials were confirmed in 38 (17.8%) samples, and reveals that a portion of feed was contaminated with antimicrobial substances. The most samples contained tetracyclines (73,7%), remaining 10 samples contained tylosin and enrofloxacin. The explanation for the relatively low concentrations of antibiotics found in the samples would be cross-contamination, which could occur either during manufacturing of medicated feed, or during transport to farms, or even at the farm itself (storage, manipulation, mixing operations). Higher contamination could occur due to non-medicinal use of antibiotics (prophylaxis, growth promotion).

## 201 Extended Analytical Tolerances (eAT) – a pragmatic extension of the analytical tolerances system to estimate inter-laboratory measurement uncertainties in animal feed analysis

Jens Schönherr, Andy Scheffer, Benedikt Brand, Anja Töpfer, Jürgen Danier

The system of "Analytical Tolerances for feed analysis" (AT) was established in the 1970's by the VDLUFA to provide a tool for harmonized assessment of animal feed analysis results. It is based on reproducibility data from VDLUFA ring trials in feed and has been constantly improved, extended and reviewed by incorporating additional ring trial data. The AT derivation was published in the "Guideline for the derivation of AT" ([www.vdlufa.de](http://www.vdlufa.de)) to provide transparency. This pragmatic model for deriving inter-laboratory measurement uncertainties for harmonized evaluation of results of animal feed analysis is applied in german official feed control assessment of analytical results of additives and undesirable substances, with regard to product specifications and maximum or minimum levels as prescribed by the responsible authority. However, AT have yet been derived only for analytes and concentration ranges with data available from VDLUFA ring trails. In order to further estimate AT for currently undescribed analytes and concentrations the model of "Extended AT" (eAT) was developed. Unlike AT, eAT are not derived directly from ring trial data. Rather, they are justified by comparative analysis of VDLUFA ring trail performance data with recognized statistical models for the advance estimation of the reproducibility standard deviation of ring trails (HORWITZ equation and its modifications by THOMPSON). The restrictions of the Horwitz- and Thompson equations are respected and eAT are not calculated for methods, merely based on analytical convention, and only for analytes with mass-related units (or units that can be converted into such a unit). The eAT postulates reasonably expected AT for concentrations and parameters, currently not directly validated through ring trials. It can be calculated as follows:

Analyte unit	Concentration (c)			eAT (±)		
	<	0,000012	%	88	% relative	
%	0,000012	-	13,8	%	$0,16 \cdot c^{0,8495}$	%
	>	13,8	%	$0,4 \cdot c^{0,5}$	%	
	<	0,12	mg/kg	88	% relative	
mg/kg	0,12	-	138.000	mg/kg	$0,64 \cdot c^{0,8495}$	mg/kg
	>	138.000	mg/kg	$40 \cdot c^{0,5}$	mg/kg	
	<	120	µg/kg	88	% relative	
µg/kg	120	-	138.000.000	µg/kg	$1,81 \cdot c^{0,8495}$	µg/kg
	>	138.000.000	µg/kg	$1265 \cdot c^{0,5}$	µg/kg	

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## 202 Feed adulteration with silkworm: detection via DNA and protein

Stobernack, T., Zarske, M., Schmolke, S., Niedzwiecka, A., Herfurth, U., Braeuning, A., Zagon, J.

In Europe, seven defined insect species have been authorized for feeding in aquaculture recently (REG (EU) 2017/893).

So far, the positive list does not contain the silkworm *Bombyx mori*. Silkworms are produced in vast amounts especially in China and India, the most important silk producing economies world-wide. Manufacturing one kilogram of silk yarn leaves roughly six kilogram pupae as by-product. Dried silkworm pupae are rich in protein and unsaturated fat and are thus an attractive substitution for higher priced fish meal in feed. However, as long as silkworm protein is prohibited, official control relies on the availability of analytical methods capable to prove the absence of this species in feed. To the best of our knowledge, there are currently no methods available for this scope of investigation. Hence, the aim of our project was to set up new, routine-suited methods for reliable silkworm detection in feeding stuff. Two approaches were chosen: (i) DNA detection via real-time PCR (polymerase chain reaction) targeting single (storage protein 1, SP1) and multicopy (cytochrome oxidase, COI) genes for rapid screening purposes, and (ii) protein detection via immunoaffinity enrichment followed by liquid chromatography - mass spectrometry (LC-MS/MS). Using PCR methods, a limit of detection (LOD) for *B. mori*-DNA as low as 1 pg was determined in a series dilution in 100% background DNA from fish feed and 0.05% (w/w) silkworm powder in a fish feed sample from the market via the multicopy gene. For protein detection, peptide fingerprints were characterized from commercially processed silkworm meals in a first step. *B. mori*-specific, heat-resistant (i.e. non-modified) peptides were identified and subsequently used to raise polyclonal antibody sera. Using these for immunoaffinity enrichment prior to semi-targeted LC-MS/MS is expected to reduce the LOD from 10% (w/w) feed adulteration towards the LODs achieved with PCR. The performance of both approaches is currently assessed with various matrices and mixtures. Thus, DNA- and protein-based methods are now available for reliable detection of silkworm adulteration down to < 1% (w/w) in feed matrices.

## 203 Adulteration of high protein feed materials by urea – challenge for official control

Waldemar Korol, Jolanta Rubaj, Grażyna Bielecka

The aim of the paper was to present the results of high protein feed materials (HPFM) monitoring in the scope of the content of urea. In the years 2016-2018 total number of 166 samples, mainly soybean products, feed yeast and fish meal, were investigated in the feed monitoring program carried out by NRIAP. Urea was tested by spectrometric method, according to the Reg. No 152/2009. LOQ of the method equals 2 g/kg. Expanded uncertainty  $U$  ( $k=2$ ) of measurements equals  $\pm 1$  g/kg at the level  $\leq 10$  g/kg and  $\pm 10\%$  at the level  $> 10$  g/kg. Polish native regulation allows to trace levels of urea in HPFM equal to 5 g/kg. Therefore, urea content higher than 5 g/kg was considered as incompliant with the requirements, but only if urea was not declared on the label. In 5 samples of investigated soybean cakes, the content of urea amounting from 15 g/kg to 64 g/kg (crude protein equivalent 45-192 g/kg –  $N \cdot 6.25$ ), indicated the scale of adulteration. The content of amino acids in soybean cake was also determined and their share in total protein was from 51.0% to 66.2%, the mean value of 56.5%, while amino acids make typically ca. 95% of total protein in soybean products. In 11 samples of feed yeast urea content ranged from 6 g/kg to 106 g/kg with equivalent of crude protein from 17 g/kg to 307 g/kg. The presence of urea in HPFM has been monitored in Poland in the official feed control since 2013. The official program started after risk assessment on the bases of the NRIAP monitoring data from previous years. Total number of 160 samples are tested yearly. Number of adulteration cases has been decreasing, from 5 cases in 2013 (4.7%) to one case in 2018 (0.6%), with 1.7% on average. However mean degree of adulteration by urea in high risk HPFM in the last years of NRIAP monitoring program (2016-2018) was higher and equal to 9.6% (3.2% per year). The samples of adulterated HPFM often contain ammonium ions. This is a challenge for official control to cover possibly representative samples of HPFM.

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## 204 Analysis and differentiation of herbicide tolerant, conventional and organic soybeans by direct comparison of tandem mass spectra

Varunjikar M.S.<sup>1</sup>, Bøhn T.<sup>1</sup>, Sanden M.<sup>1</sup>, Belghit I.<sup>1</sup>, Palmblad M.<sup>2</sup>, Rasinger J.D.<sup>1</sup>

Nutritional and elemental similarity between herbicide tolerant genetically modified (HT GM) crops and their traditional non-GM counterparts are commonly used to argue that GM crops are substantially equivalent to, and therefore as safe and nutritious as, currently consumed plant-derived foods from other agricultural practices. In a study published in 2014, Bøhn et al. demonstrated "substantial non-equivalence" in 'ready-to-market' soybeans obtained from 31 individual fields/sites in Iowa, USA. In contrast to conventionally or organically farmed soy, GM-soy contained residues of both glyphosate and AMPA; in addition, the nutritional profile based on 35 different nutritional and elemental variables, could be used to discriminate GM, conventional and organic soybeans without exception (Bøhn et al. 2014). In the present study, by using high resolution tandem mass spectrometry and high-throughput bioinformatics analyses and the same 31 individual soybean samples from Iowa, we expand on this previous study. We test whether proteomic mass spectrometry profiles of GM soybeans are equivalent to conventionally and organically farmed soy samples. We also assess if the application of shotgun proteomics in combination with direct comparison against spectral libraries provides for a novel approach capable of classifying soybeans according to their origin, type and variety, as well as pre- and post-plant pesticide treatment or level of pesticide residues in the soybean product.

References: Bøhn, T. et al. Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. *Food Chem.* 153, 207–215 (2014)

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## 205 Authentication of feed – development of a spectral database

F. Wustrack, J. Raeke, C. Fauhl-Hassek, J. Riedl

Feed material is an integral part of the food supply chain. Against the background of various feed incidents, e.g. the dioxin affair 1999 in Belgium, which was caused by the illegal addition of transformer oil to feed material, the need for analytical methods for the authentication of feed becomes obvious. Especially unknown, possibly harmful substances, which are added illegally, often cannot be detected with classical, targeted methods. Therefore alternative analytical concepts need to be developed. For this purpose non-targeted analytical methods using spectroscopic or spectrometric techniques and subsequent multivariate data analysis are suited. For these approaches databases that describe the typical composition of the feed material are needed. These reference data can then be used to detect any deviations from the "normal", which might be an indication for an adulteration or contamination. The final aim of this project is the development of an expandable and versatile tool for the food and feed control laboratories, which provides spectral information on different feed material and can be used for authentication and the identification of potential risks.

In previous studies the verification of the geographical and botanical origin of distillers dried grains and cereals (DDGS) and grain maize as well as the detection of adulterants in edible oils were studied [1, 2, 3]. The results will be summarized shortly. These studies were proofs-of-principles relying on data sets limited to certain time periods and geographical or botanical origins. The missing concepts for validation and long-term maintenance of the methods are current challenges that hinder routine applications. To address these challenges a concept for a reference database is developed using existing data sets from previous studies. Furthermore, the database will be initially expanded with a focus on oils and fats for feed that may serve as reference spectra. Therefore in particular soybean oil, rapeseed oil or animal by-products, as well as selected potential adulterants (e.g. mineral oil, transformer oil) and by-products from the food production (e.g. fatty acid distillates) will be analyzed. For these analyses non-targeted spectroscopic methods will be used. By means of multivariate statistical analysis class models for the detection of suspicious samples will be developed, thoroughly validated and continuously extended.

[1] Nietner et al., 2013, *Journal of Agricultural and Food Chemistry*, 61, 7225-7233

[2] Achten et al., 2019, *Food Analytical Methods*, 12, 2172-2184

[3] Pfister et al., 2018, *NIR news*, 29, 6-11

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## 206 Inter-laboratory study on the implementation of the analytical procedure for the determination of glyceroltriheptanoate (GTH) in animal by-products: results and conclusions

O. Fumiere<sup>1,2</sup>, U. Vincent<sup>3</sup>, C. von Holst<sup>3</sup>, P. Veys<sup>1,2</sup>

As stated in Commission Regulation (EU) 142/2011, derived products from Category 1 and 2 materials must be marked with glyceroltriheptanoate (GTH). A method based gas chromatography coupled to mass spectrometry (GC-MS) for the determination of GTH in animal by-products was validated in 2010 (Boix et al., 2010). The European Union Reference Laboratory for Animal Proteins in feedingstuffs (EURL-AP) was mandated by the European Commission to conduct an inter laboratory study to assess the implementation of the method in laboratories designated by the competent authorities of the Member States. An additional objective of this study was to evaluate whether a revision of the protocol would be required and if the method as such is suitable for GTH detection in feed matrices.

Nineteen laboratories participated to the study and received a sample set consisting of 6 fats representing a sub-group on animal by-products marked or not with GTH. One of the samples was an industrial Category 2 fat already marked with GTH; the five remaining samples were prepared starting from an industrial Category 3 fat, free of GTH. Knowing that the GTH target concentration in marked materials has to be at least of 250 mg per kg of fat, samples were prepared according to a 5 levels of marker design (0, 25, 50, 100 and 250 mg/kg respectively). The final assigned values of 0, 23, 46, 91 and 228 mg/kg respectively were calculated according to the formulation, taking into account the purity of GTH and assessed by comparison with the values for the content of GTH in the different samples determined during the homogeneity study.

The participants' results were generally quite good and showed a good implementation of the method. For some few labs, investigations are needed to explain their deviating results. The study as well as the results and conclusions will be completely presented.

Keywords: Animal by-products, GC-MS, GTH, feed

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## 207 Characterization of hydrolysed proteins as feed ingredient

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The production of food automatically results in a range of by-products, ranging from post-harvest to post-retail. European legislation prohibits direct recycling of animal proteins by means of the species-to-species ban.

A major legal category is defined as Processed Animal Proteins (PAPs). Materials exempted from this definition are not subjected to the species-to-species ban. Most hydrolysed proteins (HP) are excluded from the definition of PAPs (Regulation (EU) 142/2011, Annex I, 5). The European Commission is currently in a process of establishing thresholds for parameters characterising hydrolysed proteins. The most important parameter is the size of the protein fragments. As yet, hydrolysed proteins derived from ruminants should have a molecular weight below 10 kDaltons.

Standard methods for the detection and characterization of PAPs cannot be applied to a range of different animal by-products and by consequence to HP. Therefore, a consortium of the NRLs from the Netherlands, France and Poland (WFSR, SCL, PiWet) has invested in the development of monitoring methods for hydrolysed proteins. These methods are based on (total or protein) solubility, SDS-PAGE and Size Exclusion Chromatography. Parameters for a definition of hydrolysed proteins might be based on a minimum (protein or sample) solubility in either water or a buffer, and a minimum portion of proteins smaller than 10 kDalton.

In the presence of several methods for monitoring different types of processed animal proteins, an approach for the distinction between animal by-products should be available in order to apply the correct method(s) and to collect the appropriate parameters. Concepts for this desired approach will be presented and explained.

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## 208 Identification of ovine processed animal protein in feeds

Anna Weiner, Ilona Paprocka, Krzysztof Kwiatek

In the past, various types of meal, for example: meat-bone, meat, bone, blood, skins obtained from animal origin were an important source of valuable protein in the nutrition of farm animals. Restrictions on the use of meat and bone meal (MBM) as feed material were introduced by the European Commission as result of the BSE crisis. In accordance with regulations, the use of processed animal protein from ruminants in feeding farm animals is banned. It caused a necessity of development of new methods allowing identification and discrimination of specific proteins. In the present work, a specific PCR-based procedure was optimized in the laboratory for identification of ovine DNA in feeds.

Examinations were performed applying bovine MBM and fortified compound feeds with MBM of ovine origin. From tested samples DNA was isolated with the commercial test (Wizard Magnetic Purification DNA System for food, Promega). Examinations were performed with fortified ovine MBM compound feedingstuffs on levels 0.5 %, 0.2 %, 0.1 %, and 0.05%. The characteristics of primers were used for identification. Parameters of the amplification were drawn up. Obtained products of amplification were subjected electrophoresis in 2% agarosis gel in TBE buffer, at permanent voltage 110 V. In the study the limit of detection for PCR was determined on 0.05 % for ovine proteins. In the validation study the accuracy was 97.8%, sensitivity – 97.1% and specificity – 100%.

A possibility of achieving correct results irrespective of the presence of elements without the characteristic morphological structure is an additional advantage of the PCR method. Based on obtained results of the validation studies were found that the PCR method can be used in routine tests for identification of ovine DNA in feeds.

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## 207 Possibility of use SDS-PAGE method for the differentiation of insect proteins

Weiner Anna, Paprocka Ilona, Kwiatek Krzysztof

Fishmeal has been the usual source of animal protein to feed farmed animals. The decrease in availability and increase in the prices of fishmeal incline to search alternative of animal protein. Interest in insects is currently increasing as an alternative source of protein in animal nutrition. Insects can complement feed materials such as soy, corn, cereals or fishmeal. Commission Regulation (EU) 2017/893 since 1 July 2017 authorised seven insect species for aquaculture. It caused a necessity of development of new methods allowing identification and discrimination of specific proteins. We checked the possibility of differentiating two species of insects: *Tenbrio molitor* and *Hermetia illucens* by SDS-PAGE.

The separation of proteins based on molecular weight, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used. SDS anions binds to hydrophobic groups of amino acids giving them a negative charge, which allows reducing the non-covalent interactions that occur in native proteins. Separation of the proteins is carried out on polyacrylamide gels forming a kind of permeable sieve, depending on the density of the molecule with different molecular masses. Based on analysis of different samples, the SDS-PAGE method could be a useful tool for distinguish two species of insects.

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## 210 Quantitative Immunoaffinity-Based Mass Spectrometry for Proving the Species and Tissue Authenticity in Feed

Steinhilber AE, Planatscher H, Zagon J, Braeuning A, Lampen A, Joos TO, Poetz O

Processed Animal Proteins (PAPs) are considered as a sustainable protein source to improve the nutritional profile of feed for livestock and aquaculture. However, the use of these proteins is strongly regulated since the bovine spongiform encephalopathy (BSE) crisis. The reintroduction of non-ruminant PAPs for use in aquaculture in 2013 has driven the need for alternative analytical methods to determine the species origin as well as the tissue source (legal or not). The current official methods, light microscopy and polymerase chain reaction, do not fulfill these requirements. Furthermore, future methods need to be quantitative, because the pending zero-tolerance-concept is planned to be replaced by accurate thresholds.

To address this issue, we developed and validated two multiplex mass spectrometry-based immunoassays together with a new sample preparation procedure named heterogeneous phase digestion (HPD). The workflow comprises a direct tryptic digestion of PAPs in suspension via HPD, an immunoaffinity enrichment of released peptides, and a LC-MS/MS-based analysis for peptide quantification using isotope labeled standard peptides. One multiplex immuno-MS assay uses a group-specific antibody that recognizes homologous peptides from the 9 livestock species' alpha-2-macroglobulin: cattle, sheep/goat, pig, horse, turkey, goose, chicken and duck. Another multiplex immuno-MS-assay uses 7 peptide-specific antibodies that recognize ruminant-specific peptides from the tissue types meat, bone, cartilage, blood and milk.

The assay performance was tested on reference mixtures obtained from our project partners. The method showed a sensitivity of only 0.1% (w/w) PAP which is the targeted sensitivity of alternative methods. Both, a high background of another species (porcine blood) and legal bovine milk powder did not interfere and were specifically detected. The relative composition of unknown samples could be estimated using QC samples.

The developed assays show high performance regarding sensitivity, quantification capabilities and species and tissue specificity. The assays are planned to be further developed and optimized. A higher number of reference samples has to be analyzed to enable quantification in unknown samples without the need for QC samples.

## 211 Feed fraud vulnerability assessment and prevention plan in compound feed production

Yilmaz E.

Consumers' trust in the food industry and industrial foods has declined with the increase in the adulteration on food in recent years. The problem of trust in the food industry has caused losses also to businesses that do their job properly. This problem of trust has prompted various platforms and GFSI (Global Food Safety Initiative) institution has been established by the Consumer Products Forum (CGF) and certification programs such as GFSI approved BRC, IFS, FSSC 22000 have been formed to increase people's trust in industrial foods. One of the issues that all three of these certification programs deal with as priority is the risk of deliberate food and feed fraud (imitation and adulteration). According to the GFSI agency, Food Fraud is a collective term for deliberate substitution, addition or misrepresentation of food, feed, food ingredients and packaging for economic gain. Adulteration is "production of food and feedstuffs, substances and materials in contact with food, in violation of the legislation or permitted features." Imitation is "imitating food and feed materials and also the substances and materials in contact with food as having the characteristics that they don't really have in terms of their form, composition and quality." Unintentional feed safety hazards are being addressed with HACCP plans. However, this does not work in deliberate actions. In addition to HACCP plans, fraudulent and adulterous dangers, especially by manufacturers and suppliers, should be considered. First, a feed fraud vulnerability assessment should be carried out covering raw materials purchased and suppliers. The organization's feed fraud reduction plan should be supported by its feed safety management system. A risk assessment can be made for each feed raw material using the following food fraud vulnerability assessment method. Scoring is done using the method given for each vulnerability criterion and the risk value is calculated by multiplying the scores given. According to the calculated risk value score, action planning is made based on the table below. In this way, we ensure feed safety by creating a feed fraud evaluation plan in the feed industry as well as in food safety.

Keywords: feed fraud, feed safety, processes, feed production

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## 212 Laboratory experience with microscopic method for detection of insects PAP

Anna Weiner, Ilona Paprocka, Krzysztof Kwiatek

There is growing interest in insect as a new protein source for animal protein. Insect are part of natural diet of many fish species and free-range hens. Insects can complement feed materials such as soy, corn, cereals or fishmeal. Commission Regulation (EU) 2017/893 since 1 July 2017 authorised seven insect species for aquaculture. We checked the usefulness of the modified microscopic method with double PE/TCE sedimentation protocol for detection of insects in feed.

In the first stage, samples of insect proteins were examined. On the basis of the obtained microscopic images, the characteristic features were determined. In the second stage of the project, 30 samples of poultry feed (10 samples of starter, 10 samples of grower and 10 samples of finisher) with the insect PAP were analyzed. Each type of poultry feed with 0%, 13%, 20% and 27% of insect PAP were spiked. The microscopic examination with double PE/TCE sedimentation protocol revealed the presence of elements typical for each insect species.

The obtained results demonstrated that using microscopic method with double PE/TCE sedimentation protocol it is possible to detect the presence of different materials of insects origin.

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## 213 Development of two analytical methods for urea determination in feed using LC-MS/MS and HPLC with fluorescence detection

Marco Krämer, Hildburg Fry, Oliver Kappenstein

Urea is authorised in the European Union (EU) as feed additive for ruminants. Because of its high molecular nitrogen content, it is a substance for potential protein adulteration in non-ruminant feed. The EU prescribes a spectro-colorimetric method as official control method for the determination of urea in feed, whereas the Association of Official Analytical Chemists (AOAC) in the United States recommends an enzymatic method. Discrepancies between results obtained by these different approaches have been reported in literature, especially at low concentrations and in matrices like pet food.

We developed and in-house validated two methods for urea determination over a wide concentration range in compound feed, including pet food, and yeast (*Saccharomyces cerevisiae*) using LC-MS/MS and HPLC with fluorescence detection (HPLC-FLD).

Limit of quantification is below 10 mg/kg for LC-MS/MS and HPLC-FLD. Variation coefficients for both methods did not exceed 10 % for ruminant feed as well as spiked samples of complete feed for chicken, pet food for dogs and cats as well as yeast. Recovery rates for spiked samples ranged from 86 to 105 %. In samples of complete feed for poultry, wet and dry pet food for cats and dogs and yeast we determined amounts of urea between < LOD and 200 mg/kg relative to a moisture content of 12 %. Results between LC-MS/MS and HPLC-FLD were in very good accordance.

The newly developed methods proved to be a suitable approach to detect urea over a wide concentration range in different feedstuff. With their quick and easy sample preparation, they represent an interesting supplement for official feed control in the future. Because of its' comparatively less expensive equipment and broad availability, HPLC-FLD might be used for routine analysis, whereas LC-MS/MS might serve as a means for confirmation of questionable findings.

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### 301 Nutritive value and fermentation characteristics of whole plant sorghum silage from different sorghum varieties

Terler Georg, Resch Reinhard, Gappmaier Stefanie

In Middle Europe, the occurrence of the Western corn rootworm (*Diabrotica virgifera*) and dry periods led to increasing damages in maize cultivation. Therefore, whole plant sorghum silage (WPSS) is being discussed as an alternative to forage maize in cattle feeding. However, so far, there is little information about the nutritive value of WPSS in Middle Europe. Thus, nutrient composition, fermentation characteristics and whole tract digestibility of WPSS were examined and compared with forage maize in a tri-annual trial (EIP project "Innobrotics"). The study included six sorghum varieties differing in predominant use: ES Aristos (BS – biomass sorghum), ES Harmattan, RGT Vegga, NutriGrain, Primsilo (SS – silage sorghum) and RGT Ggaby (GS – grain sorghum). Plants were harvested at three different maturity stages of the grain (late milk stage, dough stage, physiological maturity). Furthermore, a forage maize variety (Angelo) was tested as reference. BS had low panicle proportion (10.5%) and therefore high neutral detergent fibre (NDF) content (616 g/kg dry matter [DM]) and low starch (XS) content (68 g/kg DM). The NDF content declined and the XS content rose with increasing panicle proportion (59.1% panicles, 458 g NDF/kg DM and 277 g XS/kg DM in GS, SS was intermediate) resulting in elevated whole tract digestibility and energy content. Compared to forage maize (10.7 MJ ME/kg DM), the metabolisable energy (ME) content of WPSS was 1.2 (GS), 2.0 (SS) and 3.3 MJ ME/kg DM (BS) lower. The optimal harvest date was the dough stage as NDF content decreased and XS content increased until this grain maturity stage, resulting in highest whole tract digestibility and ME content. Silages from SS varieties had leachate losses which were due to low DM content of WPSS (< 250 g/kg fresh matter). Drop of pH during fermentation was insufficient in several WPSS and high ethanol content was found especially in silages from BS and SS varieties. The lower nutritive value is an argument against the use of WPSS instead of forage maize in cattle feeding. However, especially in temperate regions with water stress and poor soils or high occurrence of the Western corn rootworm, WPSS can be an interesting alternative.

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### 302 Assessing the contamination with mycotoxin mixtures and phytoestrogens of the diets in selected dairy farms in Austria

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Mycotoxin contamination of feeds can trigger a reduction of animal welfare and productivity, resulting in economic losses as well as posing health risks for consumers of contaminated foods. Research, not only on regulated but also on "emerging" and "masked" mycotoxins, in agricultural commodities and the combinatory effects of mycotoxin mixtures is necessary to understand the toxicological impact of these mixed metabolites. Additionally, phytoestrogens may induce negative impacts on the reproduction of ruminants. Multi-metabolite assessments via large-scale epidemiological studies are essential for risk assessment. This study aimed to determine a wide-spectrum profile of mycotoxins and other secondary metabolites in the diets of Austrian dairy cows. Representative samples of lactating cows' diets (n=198) were collected in duplicate from 100 dairy farms from Styria, Lower and Upper Austria during May 2019 and September 2020. The samples were dried at 65°C for 48h, milled (final particle size: of <0.5mm) and analysed using LC-MS/MS (Spectrum 380®), which covered over 800 compounds at the time of analysis. In total, 159 different mycotoxins, other fungal metabolites (from *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium* and others, including ergot alkaloids), phytoestrogens and other metabolites were detected. Samples were co-contaminated ranging from 30 to 72 metabolites/sample (average:48). Deoxynivalenol, zearalenone and fumonisin B1 were the most recurrent regulated mycotoxins contaminating 92, 78 and 71% of the samples, respectively. The most prevalent emerging mycotoxins were beauvericin, enniatins, emodin and culmorin. As estimated at the average DMI of 22 kg/cow/day, the mean intake of mycotoxins, phytoestrogens and other metabolites would be 2115 mg/cow/day (ranging:333–9592). Even if the individual levels of mycotoxins did not show high concentrations, complex mixtures of mycotoxin/endocrine disruptors (e.g., phytoestrogens) are a persistent and underestimated risk for dairy farming. The present data foster further research to elucidate relationships between dietary contamination and health, fertility, and productive performance of dairy cattle herds.

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## 401 The analysis of animal faeces and liquid manure as a tool to monitor antibiotic usage in livestock

Ewelina Patyra, Monika Przeniosło-Siwczyńska, Zbigniew Osiński, Tomasz Grenda, Krzysztof Kwiatek

Veterinary antibiotics are used globally to treat diseases and to protect the health of animals. Most pharmaceutical active ingredients can be excreted by the medicated animals, and depending on the structure up to 90% of the initial compound can be found in the urine and/or faeces afterwards. However if animals are treated, residues of the antibiotic substances can frequently be detected in manure due to poor absorption of the respective substance in the animal gut or unmetabolized extraction. Manure/slurry is characterized by a high content nutrients making it valuable as a soil fertilize. In this paper, a simple and robust method is proposed for the simultaneous analysis of oxytetracycline, tetracycline, chlortetracycline, doxycycline, enrofloxacin, ciprofloxacin, trimethoprim, tiamulin and tylosin from faeces, liquid manure and digestate. These extraction methods utilize a range of different extraction solvents and are generally based on mechanical shaking, ultrasonication or vortex mixing. The sample preparation was done by using ultrasonic extraction with McIlvaine-Na<sub>2</sub>EDTA buffer solution and purified by SPE (Strata-X-CW cartridges) and analysed by HPLC-MS. Validation of the method was performed according to the guidelines indicated in European Commission Decision 2002/657/EC. Recoveries from spiked pig and poultry faeces and liquid manures samples were ranged from 76.1 to 112.0% depending on analytes. Quantification limits of the method were measured from 15.1 µg/kg for trimethoprim to 72.3 µg/kg for tylosin.

This study investigated the level of antibacterial contamination in twenty six pig and poultry faeces, four liquid manure samples and eight digestate samples were collected from different sampling areas located in Poland. The residue levels of selected fluoroquinolones, tetracyclines, trimethoprim, tylosin and tiamulin in 26 animal faeces (swine and poultry), 4 liquid manure samples and 8 digestate samples collected in 2018 from large-scale livestock and biogas plants in Poland. The analysis of 38 samples revealed that 13 samples (34.2%) were positive for the presence of doxycycline, enrofloxacin, oxytetracycline and tiamulin. The obtained results in the presented study demonstrated that veterinary antibacterial substances can lead to the contamination of agricultural soils via fertilization with animal manure.

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Analytical chemistry, antibacterial substances, feed, faeces, feed additives, HPLC, DAD, FLD, MS, solid phase extraction, QuEChERS, validation

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## 402 ZenA renders zearalenone non-estrogenic in pigs

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According to the global BIOMIN mycotoxin survey, 48 % of 19,007 grain samples analyzed between January and December 2020 were contaminated with the Fusarium mycotoxin zearalenone (ZEN). In pigs, the species considered to be most sensitive to this mycotoxin, ZEN negatively affects reproduction and evokes symptoms of hyperestrogenism. The present 42-day study aimed to prove the ability of the novel enzyme zearalenone hydrolase ZenA (ZENzyme®) to biotransform ZEN into the non-estrogenic metabolites HZEN (hydrolyzed ZEN) and DHZEN (decarboxylated HZEN) within the digestive tract of piglets. A total of 36 weaned piglets (mixed sex) were split into three experimental groups with three pens per group. A control group received an untreated, uncontaminated grower diet; the second group was fed a diet contaminated with 200 µg/kg ZEN; the third group received feed contaminated with 200 µg/kg ZEN and supplemented with 10 U/kg ZenA. Performance parameters were recorded on a regular basis. ZEN and its metabolites HZEN, DHZEN and alpha-zearalenol (α-ZEL) were analyzed as biomarkers of exposure in feces on days 21 and 42 of the trial. As expected, no significant differences were observed between the groups with regards to growth performance. Upon administration of ZenA, concentrations of ZEN and its more estrogenic metabolite α-ZEL were significantly reduced in feces at both investigated timepoints and the non-estrogenic metabolites HZEN and DHZEN were detected. Results suggest that the application of zearalenone hydrolase ZenA as a feed additive is a promising approach to prevent estrogenic effects of ZEN in pigs.

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### 403 The evaluation of growth performance, digestibility and blood biochemistry parameters of broilers fed based diets supplemented with essential oils mixture

Sanja Popovic<sup>1</sup>, Nikola Puvaca<sup>2</sup>, Tatjana Peulic<sup>1</sup>, Vojislav Banjac<sup>1</sup>, Danka Dragojlovic<sup>1</sup>, Sladjana Rakita<sup>1</sup>, Olivera Djuragic<sup>1</sup>

In order to investigate the potential of dietary essential oils mixture of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*), on production performances, digestibility and blood biochemistry parameters of broilers, biological trial was conducted. The investigation was carried out under production conditions on a total of 840 day-old Ross 308 broilers which were equally distributed into three dietary treatments with eight replicates each. In the control treatment (C) broilers were fed with basal diet, while in experimental treatments (E5 and E10) broilers were fed basal diets supplemented with essential oils mixture in concentrations of 0.05 and 0.10%, respectively. Thyme, oregano and rosemary essential oils were present in mixture in concentrations of 4, 3 and 3 g/100 g, while calcium carbonate was used as a carrier. The added essential oils mixture showed its positive properties as a growth promoter and influenced more intensive growth of broilers, as well as significantly lower feed conversion ratios in experimental, compared to control broilers. Additionally, the highest European production index of broilers was recorded in the treatment E5 (298.23%), while in the treatment E10 a slightly lower value of this indicator was achieved (289.21%). The added mixture of essential oils did not influence lipid profile status of broilers blood. Moreover, added natural growth promotor had positive effect on the digestibility of proteins, fats, ash, calcium and phosphorus in broilers, and thus increased the utilization of basic nutrients. Based on the obtained results, it can be stated that usage of well-balanced feed, which contains optimal content of thyme, oregano and rosemary essential oils mixture, is completely justified in broilers nutrition.

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### 404 Fruit and vegetable by-products as functional feed ingredients in animal nutrition

Sladjana Rakita, Dušica Čolović, Vojislav Banjac, Ivana Čabarkapa, Olivera Đuragić, Nedeljka Spasevski, Sanja Popović

For most fruits and vegetables, the generation of by-products is estimated to be around 30% of the processed material. Fruit and vegetable by-products have been recognised as promising sources of valuable substances such as phytochemicals (phenolics, flavonoids, and carotenoids), vitamins, antimicrobials, or dietary fats that have beneficial technological activities or nutritional properties. Fruit and vegetable by-products are good sources of natural antioxidants due to their high phenolic content. Grape by-products, red beet by-products, apple pomace and potato peel are rich in polyphenols. Carrot peel and tomato skin are good source of carotenoids. Tomato seeds are rich in unsaturated fatty acids. By-products of fruit and vegetable processing and their chemical constituents with bioactive potential offer numerous promising possibilities as tools for improving animal production by inclusion in the diet. Tomato pomace is a significant source of lycopene, a pigment that gives colour to meat. Lycopene is a known antioxidant and can help in reducing oxidative stress in ruminants. When included in monogastric nutrition, grape pomace as a source of polyphenols may improve certain aspects of meat and milk quality and modify intestinal microbiota. Flavonoids generally have positive effects on villi morphology and can enhance pig health by preventing intestinal inflammatory processes and improving growth performance and N digestibility. Inclusion of polyphenols in chicken diet may help in reducing meat lipoperoxidation and improving vitamin E status. Incorporation of fruit and vegetable by-products as a source of carotenoids into chicken diet enhances the egg yolk colour. However, there are several factors that limit the use of these alternative feed ingredients in the animal nutrition. Presence of anti-nutritional factors and product inconsistency due to endogenous differences in their composition, as well as the fact that these by-products are locally and seasonally produced notably affects successful commercial exploitation of the by-products. Complicated feed legislation and restrictions and the influence of added by-products on feed palatability is another factor restricting their usage. Concerning that fruit and vegetable by-products represent relatively new class of functional feed ingredients, there are little information on their inclusion in animal diet, their bioactivity and interactions with other feed compounds, and therefore should be thoroughly explored.

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### 405 Accuracy and robustness of high pressure ion chromatography with conductivity detection for the analysis of nitrate and nitrite in varying molasses matrices

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Current EU guidance values for nitrate and nitrite in feed materials necessitates their frequent monitoring in sugar beet molasses. Consequently, the application of validated, accurate and robust quantification methods is crucial. Besides photometric methods, which are quick and easy to use, the gold standard is high performance ion chromatography with conductivity detection (HPIC-CD). However, analysis performed at different laboratories with varying sample preparation and instrumental setup used (e.g. separation column, mobile phase, gradient conditions) results in poor reproducibility of results. Moreover, each sugar factory's molasses has a different matrix influencing analysis (e.g. separation, detection) and quantification, which leads to ambiguity as to whether molasses meets present guidance values. In a round robin study, five participant laboratories were first asked to analyze sugar beet molasses with varying nitrite and nitrate concentrations according to a standard operating procedure providing instructions on sample preparation, but not on instrumental settings or methods. Later, participants received molasses samples from different factories spiked with varying nitrite content in order to assess matrix effects on the results of quantitative analysis. First results showed that participants unfamiliar with handling of molasses had difficulties with sample preparation and therefore delivered strongly deviating nitrite values. On the other hand, participants regularly handling molasses, but not experienced with nitrite analysis on HPIC-CD, delivered results deviating due to instrument setup and applied methods. After analysis of the first round of samples, participants were asked to adapt their methods and, if possible, instrumental setup in order to harmonize procedures for assessment effect of the molasses matrix upon analysis and results. While nitrate levels showed low variance between and within all laboratories (i.e. high precision under reproducibility and repeatability conditions of measurement, respectively), determination of nitrite levels was repeatable within a laboratory, but showed pronounced variance between laboratories. Most importantly, the outcome of this round robin study helps to inform participants whether their results are reliable and if nitrate and nitrite values are below EU guidance values. However, considering the variance of nitrite levels between laboratories, we suggest to opt for development of a standardized HPIC-CD method in order to minimize inaccuracy.

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### 406 Application of the cutaneous hypersensitivity test to evaluate the effect of different adsorbents on the immune response of broilers exposed to T-2 toxin in feed

Ksenija Nestic, Svetlana Milanovic, Miodrag Lazarevic, Bozidar Savic, Marija Pavlovic, Vladimir Radosavljevic, Vladimir Nestic

Cutaneous test with phytohaemagglutinin (PHA) is routinely used to test the T cell response in broilers as one of the three major parameters of immune system activity. This technique is based on the measurement of skin fold after PHA injection which has good mitogenic and blastogenic properties and does not cause physiological stress. One hundred broilers were divided into five experimental groups (control group K which received feed without adsorbent and toxin, and four experimental groups which were given feed contaminated with 2 mg/kg T-2 toxin: E-I without any adsorbent, E-II received inorganic adsorbent in addition, E-III organic adsorbent and E-IV with added combined adsorbent). In order to estimate the influence of these three different adsorbents on the immune response of broilers exposed to 2 mg/kg T-2 toxin in feed, 0.1 ml of a solution containing 100 µg of PHA was injected between the 3rd and 4th digits of the left foot on the 20th and 41st days of the trial. The degree of skin thickening was measured after 24 hours. Similar results were noted for both periods. Broilers in control group (K) and experimental group (E-III) had the highest average skin fold thickness. Significant difference ( $p \leq 0.05$ ) in the treatments was noted between these groups (K and E-III) and E-I and E-II experimental groups. Immunosuppressive influence of T-2 toxin on cellular immunity was evident. Using cutaneous hypersensitivity test variable protective effect of adsorbents was demonstrated, which could be explained by the different mechanism of mycotoxin binding and/or by the influence of adsorbent components itself on cellular immunity via activation of B and T cells, as well as macrophages.

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### 407 Effect of a gut agility activator on underlying mechanisms for adaptive capacity of organs vital for resilience in chickens

Jones, G.M. and Mountzouris, K.C.

Adaptive mechanisms on the cellular level in the gut and liver of broilers were investigated in response to a gut agility activator (GAA) comprising a botanical formula. One-day-old male Cobb broilers (n=500) were allocated to 4 different treatments diets with 0, 750, 1000 and 2000 mg/kg GAA (Anco FIT Poultry) for 42 days. Each treatment was replicated 5 times with 25 birds each. Birds were euthanized at 42 days and tissue samples of liver and mucosa along the intestine were taken from 10 chickens per treatment for analysis of gene expression and 20 birds per treatment for biochemical analysis. Data were analysed by ANOVA and significant effects ( $P \leq 0.05$ ) were compared using Tukey HSD test. Polynomial contrasts tested the linear and quadratic effect of GAA inclusion levels.

Total anti-oxidative capacity (TAC) was improved in the liver ( $P=0.040$ ) and at 1000 g/kg intestinal TAC was higher in the duodenum ( $P=0.011$ ) and the ceca ( $P=0.050$ ) compared to the control. Furthermore, critical genes for enzymes belonging to the Nrf2/ antioxidant response element (ARE) pathway (SOD1, GPX2, HMOX1, NQO1, Nrf2 and Keap1) were upregulated in the duodenum and the ceca mainly in a quadratic way ( $P \leq 0.05$ ) compared to the control. Increasing GAA downregulated genes for NF-KB1 in a quadratic pattern and TLR4 and HSP70 linearly in the duodenum and ceca.

The data indicates that the GAA is positively impacting underlying adaptive mechanisms at the cellular level in the liver and certain parts of the gut which could play a role in modulating the response of birds to stressors and thus increase resilience. The effects were dependent on GAA inclusion level. Commercial applications using the effective inclusion levels of this experiment showed a positive effect on performance in the face of stressors such as heat and mycotoxins in broilers and increased laying persistence in layers at the later stages of the laying cycle. Establishing standards to evaluate resilience in poultry along with further research using the GAA under stress-challenge environments are warranted.

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### 408 Knowledge-based animal welfare discussion by integrating microbiological feed analyses - using straw as an example

Helena Karatassios (M.Sc.) und Prof. Dr. Matthias Kussin

Animal husbandry methods also play an important role in public discussion, as animal welfare is often valued in society by visual perceptions. In this context, there is often an idealized idea of livestock husbandry and nutrition, which is staged by ideal-typical images. In the minds of many citizens, nature-loving images trigger a positive imagination that results from the longings of urban living conditions. Media and stakeholder analyses indicate that the use of straw in livestock husbandry and nutrition also has a positive impact on the welfare of livestock. According to this, straw is preferred by the public for more animal welfare. But what is not considered is the fact that the straw must be of impeccable hygienic quality. Fungal infestation and the formation of mycotoxins in straw can cause diseases in livestock with consequences for animal welfare. The first evaluation of a perfect straw quality also takes place in science through sensory tests, i.e. through smell, grip, colour and impurities. Only in the case of abnormalities in the sensory tests are further examinations indicated, such as microbiological examination procedures. The hygienic properties of straw were examined on the basis of these assessment criteria. In addition to the microbiological-hygienic tests, the sensors of the straw were also tested. The results show that there are no abnormalities in the sensory examination of the hygiene status. This was to observe an impeccable hygiene status. However, the microbiological-hygienic investigations showed that the straw had microbiological as well as mycotoxin loads above the orientation values. This can have negative health effects, such as diseases for farm animals. The scientific results led to the conclusion: The public discussion about animal welfare, which is often conducted primarily on the basis of visual impressions, could gain in scientific resilience if it includes objective results such as microbiological analyses in addition to images in order to evaluate animal welfare in livestock farming.

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### 409 Efficacy of supplemented vitamin D3 and herbal calcitriol as isolated or associated sources in laying hens from week 48 to week 52 of age

M. Sc. Murat Devlikamov, Prof. Dr. Klaus Männer, Dr. Armin Müller, Dr. Anja Pastor

A common problem in the commercial layer industry is related to weak eggshells. One of the strategies for eggshell quality improvement is a supplementation with higher concentrations of vitamin D3. This fat-soluble vitamin is absorbed in the small intestine and converted to 25-OH-D3 in the liver, and subsequently to its active form of 1,25-(OH)2-D3 (calcitriol) in the kidneys. However, this process diminishes in aged laying hens and leads to a malabsorption of calcium and phosphorus in the intestine and thus to thinner eggshells and skeleton problems. An approach to overcome this issue, is dietary supplementation with the active form of vitamin D3, calcitriol, as isolated or associated source together with vitamin D3. The objective of the trial was to evaluate the efficacy of supplemented herbal calcitriol as isolated source in to diets for laying hens from week 48 to week 52 of age at graded dose levels.

The study was performed with seventy laying hens (LSL-Lite), which were selected according to age, laying status and body weight from a pool of 3,500 layers of a local breeding farm. The birds were allocated to 5 groups: 1. NC – No Additives, 2. PC 2,500 IU/D3 kg, 3. T1 (NC + 300 mg/kg herbal calcitriol product), 4. T2 (NC + 600 mg/kg herbal calcitriol product), 5. T3 (1,250 IU/D3 kg + T1). The feed was based on soybean meal, corn and wheat. Environmental and management conditions were in accordance to targets used in commercial laying hen farms.

The feed conversion ratio decreased with increasing dose levels of calcitriol in comparison to the negative control. Differences were significant regarding dose level of 130 mg/kg (-3.5%;  $p \leq 0.05$ ). With feeding the two isolated sources of calcitriol, egg production rate, egg mass and egg weight were on average 1.9, 2.7 and 1.1% greater than those recorded in the negative control ( $p \geq 0.05$ ). The eggshell breaking strength of hens fed with isolated calcitriol were significantly enhanced by 8.3% (65 mg/kg) and 12.2% (130 mg/kg) in comparison to the negative control ( $p \leq 0.05$ ).

In summary, the outcome of the current study revealed that the addition of herbal calcitriol at inclusion levels of 65 and 135 mg/kg significantly improved the averaged eggshell breaking strength. Moreover, herbal calcitriol showed positive responses on the laying performance in comparison to the negative control. Consequently, herbal calcitriol offers a valuable tool to influence eggshell quality in laying hens.

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### 410 Dietary intake assessment of selected trace elements in cattle from two different Slovenian regions

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Trace elements, inorganic constituents of feed, are either essential (necessary for the animal growth and health) or non-essential or even toxic to animals. From rations for cattle, sampled on farms in two different Slovenian regions, one contaminated (Meža valley) and one uncontaminated (Gorenjska region), dietary intake of selected elements (Al, As, Ba, Be, Cd, Cr, Co, Cu, B, Fe, Pb, Li, Mn, Hg, Mo, Ni, Se, Ag, Sr, Sn, Sb, V, Tl, Ti and Zn) and the risk of lack or excess intake in cattle were assessed.

For the calculations of element intakes in animals, 80 components of meals for cattle were collected on 12 farms the two Slovenian regions. After closed-vessel microwave digestion, inductively coupled plasma mass spectrometry (ICP-MS) was used to analyse samples. Based on the results and criteria in the literature, the lack or excess of essential elements and the levels of individual elements in rations were assessed. Further, a comparison with European legislation, which sets maximum levels of undesirable elements and maximum levels of elements added to feed was made. The contents of trace elements Cu and Se in some meals were below the required contents and the contents of Zn in some meals were low. Two meals from the polluted region exceeded the maximum permitted content of Pb, which is 5 mg/kg of complete feed with a moisture content of 12 %. High contents of Al and Fe suggested physical contamination of forages with soil. Statistically significant differences for meals from two regions were found for elements Ba, B and Cd. For seven elements (Ti, Sr, Li, Sn, Be, Sb, Ag) no criteria have been found in the literature.

The data suggest that cattle should receive mineral supplementation routinely. The most important trace elements needed are Cu and Se. Cattle fed Slovenian maize silage also should receive supplementary Zn and Co. To calculate the appropriate meals it would be necessary to analyze the ingredients of meals previously, due to the different levels of trace elements in ingredients.

Key words: animal feed; dietary intake; trace elements; ICP-MS; Slovenia

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## 411 Effects of a feed supplementation with a lignan containing lignocellulose product on the performance and gut morphometry of broilers

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**Objective:** The present study evaluates the effects of a dietary supplementation of a lignocellulose product containing lignan-rich bark (LP; agromed@ROI, premix, agromed Austria GmbH, A-Kremsmünster) on the zootechnical performance and gut morphometry of commercial broilers under field conditions in Brazil, compared to a commercial antibiotic growth promoter (AGP). Lignans are class of polyphenols which anti-inflammatory, antioxidant, and anti-microbial effects offer a potential to use the lignans in antibiotic-free feeding strategies.

**Material & methods:** In a 41-day feeding trial (SP, Brazil), 22,000 healthy day-old ROSS AP 95 broilers, fed on corn/soy based standard feed, were allocated to two treatments in two comparable stable units. In a four-phase feeding regime, the control diet was supplemented with 10 ppm Avilamycin, the diet of experimental group was supplemented with LP in an age-dependent decreasing dosage (400 ppm to 200 ppm). **Data:** performance, necroscopy of intestinal tract (d 21).

**Results:** LP supplementation increased slaughter weight by 144 grams and reduced feed conversion rate by 7 points relative to the control. The comparison of gut morphology showed a significant improvement for LP-treated birds over the control birds (increased villi height, and villi/crypt ratio; reduced crypt-depth;  $p < 0.01$ ).

**Discussion:** In this trial, the LP supplementation in a decreasing dose protocol (400g / 300g / 200g per ton of feed) boosted zootechnical performance of fast-growing broilers under commercial conditions when directly compared to birds treated with a commercially available AGP. The larger villi and higher villi/crypt ratio indicate an increased nutrient absorptive capacity for LP-supplemented birds. The reduced crypt-depth may possibly be suggestive of a reduced demand for cell renewal and less inflammatory challenge. The increase of growth performance and improved gut morphology is in line with previous findings from Potthast et. al (2018). Thus, the data indicates that LP offers a potential as an alternative to AGPs.

Literature: Potthast, C. et al. (2018): Proc. Soc. Nutr. Physiol. 27: 118

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## 412 Monitoring study on the levels of antibiotic residues in horse feed by LC-MS/MS

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Veterinary medical products are often administered by means of medicated feed, especially in livestock. Among such products there are antimicrobials, used to treat bacterial infections on a large scale. Cross-contamination, or carry over, of these drug residues in other feeds produced by the same manufacturer may occur during feed processing, storage or transport. This event is considered unavoidable. The thresholds of drug residues in non-target feed have not been established in EU to date. In Italy, the Ministry of Health Note 0012392-P-11/05/2015 states the limit of analytical action for the main classes of antibiotics at 1 mg/Kg. Whether sub-therapeutic drug residues in feed, at doses lower than 1 mg/kg, causes any effect in livestock is currently unknown. The major public health risk related to the presence of antimicrobial residues in animal feed the development of antimicrobial resistance (AMR) in animals, and its potential passage to humans. This monitoring study aims to evaluate the presence of antibiotic residues, at  $\mu\text{g}/\text{kg}$  level, in horse feeds used in the Piedmont region, Italy. Thirty-five 500 g horse feed samples (21 complementary feed and 14 ingredients) produced by EU manufacturers were collected from 18 stables located in the Turin area, and stored at  $-20^\circ\text{C}$  until analysis. A multi-residues LC-MS/MS method for the detection of 45 target antibiotics belonging to different classes of antimicrobials (tetracyclines, sulfonamides, macrolides, quinolones, pleuromutilins, streptogramins, lincosamides, diaminopyrimidines, penicillins) was employed, with a limit of detection (LOD)  $50 \mu\text{g}/\text{kg}$ , and a limit of quantification (LOQ)  $250 \mu\text{g}/\text{kg}$ . None of the tested molecules appear to exceed the LOQ in the feeds tested. Nevertheless, traces of oxytetracycline, amoxicillin, sulfadimethoxine and tiamulin were found in 3 out of 21 complementary feed samples, coming from 3 different stables and provided by 2 different manufacturers, both producing medicated feed-stuffs intended for zootechnical species. Further analyses are ongoing to improve the sensitivity of the method. Moreover, it should be investigated the possible role of these residues in selection of resistant antimicrobial bacteria in horses, and their possible transmission to humans.

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### 413 Impact of phytogenic feed additives on broiler performance under Necrotic Enteritis challenge

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**Objective:** *C. perfringens* infection caused necrotic enteritis (NE) is a common problem in poultry production, leading to performance depression and causing economic loss. Hence, increasing bird resilience against NE can have a positive effect on poultry performance. Phytogenic feed additives (PFA), consisting mainly of essential oils, herbs, botanicals or oleoresins, can have a positive influence on broilers infected with CP. The objective of this work was to investigate the impact of a PFA mixture on broiler performance under NE challenge.

**Methods:** A 42-day floor pen study was conducted with 1400 broilers (Cobb 500). Birds were allocated to four treatment groups, namely a non-infected and not-treated negative control (NC) group, an infected-but-not-treated positive control group (PC), a Bacitracin (BMD) treated group and one group treated with a PFA mixture containing Quillaja saponins and Schisandraceae essential oils. Each treatment group had 7 replicate pens with 50 birds per pen. PFA (400g/t) and BMD (50g/t) were supplemented in feed throughout the trial. The birds were infected with 10<sup>9</sup> CFU CP on D19, D20 and D21 of the study. Body weight (BW), feed intake (FI) mortality (MRT), lesion score (LS) and NE based MRT (NE MRT) were determined. LS was performed on day 21.

**Results and Discussion:** PC showed reduced performance compared to NC in terms of BW (-25,4%), ADWG (-7,7%), FCR (+4,2%) and MRT (+73,3%) throughout the trial. Additionally, PC had higher disease parameters values in terms of LS and NE MRT, indicating a challenge. Performance losses due to NE were reduced by BMD or PFA treatment. BMD and PFA numerically increased BW (+20,5; 25,3%), ADWG (+3,8%; +7,3%), FCR (-6,8%; -8,3%) and significantly decreased MRT (-77%; -82,6%) during the infection period from D21-D35 compared to PC. Furthermore, both treatments significantly decreased LS (-41,2%, -57,9%) and NE MRT (-83,7%, -83,7%) compared to PC. No significant differences were observed in any parameter between BMD and PFA, indicating that both treatments worked similarly in this trial. Thus, the tested PFA may increase growth performance in broilers under NE challenge by lessening the impact of the disease and might be a valuable tool in NE management programs.

#### Author information

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### 414 Partial substitution of DL methionine and total replacement of synthetic choline chloride by polyherbal mixtures

Angela Atkinson, Jean François Le Roux, Christophe Alleno, Céline Combeau

The aim of the trial was to test the partial substitution (50 and 75%) of synthetic DL methionine (DL-Met) by polyherbal mixture Optimethione (OPTM) and the total replacement of choline chloride 60% (CC) by polyherbal mixture Natu B4 (NB4) on broilers' performance.

The trial was conducted during finishing period (21 to 42 days of age), with 400 broilers Ross 308 (males and females). Broilers were raised from a commercial farm. At 21 days of age, 4 groups were created with 10 repetitions of 10 broilers. T100/100, positive control, CC 0.9 kg/T + DL-Met 2kg/T ; T100/50 : CC 0.9 kg/T + DL-Met 1kg/T + OPTM 1kg/T ; T0/50 : Natu B4 (NB4) 0.185 kg/T + DL-Met 1 kg/T + OPTM 0.5 kg/T ; T0/25: NB4 0.185 kg/T + DL-Met 0.5 kg/T + OPTM 0.5 kg/T. Mortality was daily recorded. Individual weights are measured at days 21, 31 and 42. Feed consumption and feed conversion ratio (FCR) are evaluated at days 31 and 42. Feathering is scored at day 42. Mortality rate was 0% and culling rate was also low (0.2%).

Weights at 42 days were not affected for both sexes together, by the different treatments. In male group, T100/50 showed a higher weight 3143g compared to positive control group 3044g

FCR was improved for both sexes together compared to T100/100, 1.80 in T100/50, 1.76, equivalent in T0/50 1.79, but degraded in T0/25, 1.84. Feather scores at day 42 were improved for both experimental groups.

This trial has demonstrated that Optimethione is able to replace 50% of DL methionine, even generating a slight increase of male body weight and improvement of Feed conversion. Associated to Natu B4, total replacement of choline chloride with half dose of Otpimethione give equivalent performance as positive control. A replacement of 75% of DL methionine and 100% of choline chloride didn't affect body weight but degrades significantly FCR. Moreover, improvements of feather score and mortality enhance animal welfare and can be explained by the diversity of actives composing the plants.

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## 415 Efficacy of a phytogenic feed additive on egg quality parameters of laying hens

Christian Stoiber, Basharat Syed, Jutta Kesselring, Mariana Masiero, Michaela Mohnl

Objectives of this study were to investigate the impact of a zootechnical, phytogenic feed additive (PFA) on egg quality parameters and general performance of laying hens over a period of 84 days. A total of 600 layers of breed Tetra SL were randomly allocated to two experimental groups, both receiving a basal diet based on corn, wheat and soybean meal. One group additionally received the PFA, a blend of essential oil components (Biomim® DC-P, BIOMIN Holding GmbH, Austria) and the untreated served as control group. Inclusion rate of Biomim® DC-P was 65 g per ton of total feed. During the trial period, starting at 22 weeks of age (1.75 kg BW) through 34 weeks of age (1.95 kg BW), mash feed and water were available ad libitum. Number of eggs, laying rate, egg mass as well as number of cracked, dirty, misshapen and unsalable eggs were recorded daily. Feed intake and feed efficiency was determined every two weeks. Every four weeks, egg quality parameters, such as haugh unit, shell/yolk color, yolk and albumen weight, shell weight/thickness/breaking strength, were assessed. Experimental unit was 10 layers per pen which was replicated 30 times per treatment group. Statistics (ANOVA, generalized linear mixed model analysis) was performed using Statistica 7.0. Initial and final BW of hens were consistent with breed standard and did not differ between treatment groups. Daily records did not show any health issues or mortality throughout the experiment. Over the entire trial period, feed intake was significantly lower in DC-P group ( $10.13 \pm 0.18$  vs  $10.24 \pm 0.09$  kg/hen,  $P = 0.005$ ), yielding improved feed conversion ratios ( $2.18 \pm 0.04$  vs  $2.23 \pm 0.03$  feed intake:egg weight,  $P < 0.001$ ). Furthermore, administration of the phytogenic feed additive increased cumulative laying performance ( $93.83 \pm 0.89$  vs  $93.30 \pm 0.7$  %,  $P = 0.017$ ), average egg weight ( $58.86 \pm 0.13$  vs  $58.64 \pm 0.14$  g,  $P < 0.001$ ) and egg mass ( $4.64 \pm 0.04$  vs  $4.60 \pm 0.04$  kg/hen,  $P < 0.001$ ). Egg quality parameters did not differ between both experimental groups. These data show that application of PFA (Biomim® DC-P) improved laying performance of hens during peak production periods.

### Author information

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## 416 Improved footpad condition – phytogenic additive shows potential for animal welfare enhancement

Iris Kröger, Elisabeth Holl, Anne Möddel, Pawel Konieczka and Monika Michalczuk

Societal acceptance of meat production and consumption today is often contingent on the producer improving animal welfare. While the focus is on management and husbandry factors, feed and feed additives also play a role. The new category for zootechnical additives in the European Union, defining physiological condition stabilisers, recognises this fact. Healthy footpads in broilers are a possible indicator for better animal welfare through improved gut health. In the present study, the effect of a hop based phytogenic additive on footpad lesions and performance parameters was tested against a positive and a negative control.

A total of 1,155 day old male broiler chicks (Ross 308) were allocated to three groups with 5 replicates and 77 broilers per pen. The broilers were kept in floor pens with wood shavings as litter material for 42 days. Broiler diets were based on wheat, soybean and maize and were fed in pelleted form. The treatment groups were either supplemented with 0.3 – 0.1 % of a prebiotic (positive control, PC) or with 0.04 – 0.02 % of a phytogenic feed additive (Anta®Phyt, PFA) and compared to the unsupplemented negative control group (NC).

Footpad condition was assessed with a recognised scoring system (Butterworth, 2009), by determining the degree of lesions advancement in a 0, 1, 2 scale. Statistical analysis was performed with SPSS 23.0. Analysis of variance (ANOVA) was used to determine the influence of treatments on production results and welfare. Chi-square test was employed to compare the frequency of footpad scores between treatment groups.

There were no statistical differences between the groups in FCR. Supplementation with the PFA increased final body weight numerically by 1 % compared to the negative and the positive control. Footpad conditions were positively influenced by the PFA. The count of healthy footpads was numerically increased in relative comparison to the NC (+1.7%) and PC (+3.2%), while the share of foot pads with minor lesions (NC: –13%; PC: –33%) and severe lesions (NC: –25%; PC: –3.5%) was reduced.

The results show that the phytogenic additive (PFA) has the potential to improve animal welfare by ameliorating the severity of footpad lesions in broilers.

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## 417 Effect of botanical feed additive on growth performance of post-weaning piglets

Tobias Steiner, Sarah Asmussen

Weaning is a stressful period for piglets resulting in feed intake reduction and growth rate depression, hence loss of performance. Botanical feed additives have shown improvements in growth performance triggered by effects on gut integrity and nutrient digestibility. The objective of this study was to evaluate the efficacy of a botanical feed additive supplemented to piglet diets.

600 barrows and female piglets (DanBred x Duroc), 26–28 days of age, with an initial BW of 7.5 kg were assigned to two dietary treatments using 30 pens/treatment with 10 piglets/pen, hence 300 piglets/treatment: (T1) Control (basal diet) vs. (T2) basal diet supplemented with Sangrovit® (200 g/t). Piglets were fed ad libitum a mesh pre-starter (0–14 days) and starter diet (14–42 days) based on wheat, barley, soybean meal and corn, containing acidifiers and phytase.

Piglets were weighed individually at 1 and 42 days to calculate the average daily gain (ADG). On day 14 piglets were weighed per pen. Feed intake was measured per pen. Faecal scores were determined daily per pen using a scale of 0 = normal, 1 = soft feces, 2 = mild diarrhea, 3 = severe diarrhea. All parameters were subjected to ANOVA and means were compared using the Tukey test.

Supplementation with the botanical additive increased BW at 14 and 42 days ( $p < 0.05$ ). Final BW in T2 was 8% higher compared with T1 (29.7 vs. 27.6 kg). Average daily feed intake in the pre-starter and starter period was 14 and 8% greater, respectively, when piglets were T2 diets. ADG was higher in the pre-starter (T1: 314 vs. T2: 370 g/d,  $p < 0.01$ ), starter period (T1: 560 vs. T2: 609 g/d) and overall. FCR did not differ between treatments but tended to be lower in pigs fed T2 diets (T1: 1.444 vs. T2: 1.432,  $p > 0.05$ ). Diarrhea scores were below 1 indicating no major intestinal issues, however scores were lower in T2 vs. T1 ( $p < 0.05$ ).

Dietary supplementation with the botanical feed additive is effective in improving post-weaning feed intake and ADG of piglets, while maintaining feed efficiency and supporting faecal consistency.

## 418 Effect of a plant-based feed additive on growth performance and blood biochemical parameters in Ross 308 broilers

Tobias Steiner, Juliane Dohms, Marta I. Gracia

Plant-based feed additives have shown improvements in growth performance triggered by effects on gut integrity (Kikusato et al., 2020) and intestinal parameters (Xue et al., 2017; Khadem et al., 2014). The objective of the experiment was to determine the efficacy of a plant-based feed additive on performance and blood biochemistry of broilers up to 42 days of age.

2,688 day-old male Ross 308 broilers were randomly assigned to 7 treatments with 16 pens/treatment and 24 birds/pen. A plant-based feed additive (Sangrovit® Extra, Phytobiotics GmbH) was added to the basal diet at 0 (T1), 45 (T2), 60 (T3), 90 (T4), 120 (T5), 150 (T6) and 3000 mg/kg (T7). T7 is considered an overdosage. Birds were fed a mash starter (0–21 days) and grower diet (22–42 days) based on corn, wheat and soybean meal. Performance parameters were determined at 21 and 42 days and haematological and blood biochemical parameters (2 birds/pen) were determined at 42 days. All parameters were analysed by one-way ANOVA using the General Linear Model function in SPSS. Multiple comparisons between treatments were executed using the Tukey test. Significant differences were declared at  $p \leq 0.05$ .

Compared with T1 (1.651), all supplementation levels improved overall mortality-corrected FCR by 5–7% ( $p < 0.0001$ ). Mortality-corrected ADG was increased by 5% in T2 and T4 ( $p < 0.005$ ). The European Production Efficiency Factor (EPEF) was improved by 14, 12 and 10% in T2, T4 and T5, respectively ( $p = 0.0006$ ). Overall mortality ranged between 1.0 and 2.1% and did not differ between treatments ( $p = 0.898$ ). There were no major effects on blood biochemical parameters. All haematology and clinical blood chemistry values were within reference ranges for healthy control broiler chicks.

Supplementation with the plant-based additive is effective in improving growth performance and particularly feed efficiency in broilers, thus representing a dietary strategy to improve productivity in commercial poultry meat production. Furthermore, all zootechnical and blood data support that dietary supplementation with the additive is well tolerated by broilers up to 3,000 g/t.

## 419 Composition of colostrum and serum in primiparous and multiparous sows, and fecal immunoglobulin concentration in their offspring

Lukasz Grzeskowiak, Eva-Maria Saliu, Beatriz Martinez-Vallespin, Anna Grete Wessels, Wilfried Vahjen, Juergen Zentek

**Objective:** Colostrum provides newborn piglets with essential nutrients and bioactive compounds. However, there are inconsistent data on the colostrum composition in primiparous and multiparous sows [1, 2]. The objectives of this study were to analyse whether the composition of colostrum and serum differs between primiparous and multiparous sows, and whether the fecal immunoglobulin content in their offspring differs during the suckling and post-weaning periods.

**Methods:** Fourteen primiparous and six multiparous sows were fed experimental gestation and lactation diets based on barley, wheat and soybean meal. The diets were iso-caloric and iso-nitrogenic. The sows were fed restrictively during gestation, while ad libitum during lactation. Colostrum was collected within 10 hours and serum within 16 hours post-partum. Fecal samples from piglets were collected on day 2, 6, 10, 14, 21, 35 and 42 of life and at weaning. Colostrum was assessed for crude protein and crude fat (proximate analysis), lactose (enzymatic assay), immunoglobulins (IgA, IgG, IgM) (ELISA), free amino acids and urea (HPLC), and biogenic amines (ion-exchange chromatography). Sows' serum and piglets' feces were assessed for immunoglobulins. Data were analysed by Mann-Whitney U test (significance at  $p \leq 0.05$ ; SPSS version 24.0.0.0). The animal trial was approved by the Regional Office for Health and Social Affairs (LAGeSo Reg. G0112/19).

**Results:** Comparison of colostrum from primiparous and multiparous sows did not yield any significant differences ( $p > 0.05$ ) in concentrations of nutrients, urea, biogenic amines or immunoglobulins. Serum immunoglobulins did not differ between primiparous and multiparous sows ( $p > 0.05$ ). Specific IgG-anti-Clostridioides difficile toxin A-antibodies were higher in colostrum and serum of multiparous vs. primiparous sows ( $p = 0.062$  and  $p = 0.005$ , respectively). Fecal immunoglobulins in piglets showed significant differences ( $p \leq 0.05$ ) depending on the age of the piglets and the parity of their mothers.

**Discussion:** Parity had no impact on nutritional and immunological composition of colostrum and on serum immunoglobulins. However, the gestational age may be important determinant of the specific antigen exposure and immune priming in sows, which may enhance piglet passive protection and active immunity development later in life. This may influence the piglet immune capacity and resilience, which could contribute to a better health.

### Author information

Profession: PhD Research Scientist; Experience: Gut microbial ecology, Host-microbe cross-talk, Clostridioides difficile

## 420 Feeding sows diets rich in sugar beet pulp or lignocellulose during gestation and lactation has no impact on immunoglobulin levels in sow serum and colostrum but in piglet faeces

E.-M. Saliu, B. Martínez-Vallespín, A.G. Wessels, W. Vahjen, J. Zentek, Ł. Grzeškowiak

**Objective:** Diet influences the colostrum composition and immunoglobulin content. Colostrum immunoglobulins are crucial for neonatal piglets, supporting their immune development in early life [1]. The aim of this study was to evaluate the impact of different fibre sources fed to sows on immunoglobulin concentrations in colostrum and serum and in offspring's faeces.

**Methods:** Sows ( $n=20$ ) were randomly allocated to two different feeding groups, either high in sugar beet pulp (SBP,  $n=10$ ) or lignocellulose (LNC,  $n=10$ ), both iso-caloric and iso-nitrogenic. Total dietary fibre contents were 244 and 294 g/kg DM during gestation, and 249 and 291 g/kg DM during lactation for SBP and LNC, respectively. Post-partum, serum and colostrum were collected from sows. Fresh faeces were obtained from piglets on day 2, 6, 14, 21, 35 and 42 post-partum and at weaning. Weaned piglets were reared on flat decks (4 animals/unit). Commercial ELISA kits were used to determine IgA, IgM and IgG in all specimens. Statistics were calculated using Mann-Whitney-U and Friedman tests ( $p \leq 0.05$ ) (SPSS). The animal trial was approved by the Regional Office for Health and Social Affairs (LaGeSo Reg. G0112/19).

**Results:** Colostrum and serum immunoglobulin concentrations did not differ between the studied sows. IgG accounted for the highest immunoglobulin concentration in colostrum and serum of sows, while IgA concentrations were the most abundant immunoglobulins in faeces from piglets, both independent of the diet. Faecal immunoglobulin concentrations decreased as the piglets aged. Dietary treatment had an impact on faecal IgA concentrations on days 6 and 35. IgM concentrations were significantly higher in 2-, 21- and 35-day-old piglets from SBP-fed sows, while a higher IgM concentration was observed in 6-day-old piglets from the LNC-fed group. No differences in IgG concentrations were observed between the study piglets.

**Discussion:** Low and high fermentable fibres influenced immunoglobulin concentrations in piglets' faeces in a different manner. As the colostrum immunoglobulins were unaffected, other factors must have been involved in immunoglobulin differences observed in piglets. Theoretically, fibres have a potential to influence the faecal microbial composition of the sows, which consequently might have affected the production of immunoglobulins in the piglets. However, further analysis are warranted.

[1] STOKES, C.R. (2017): J Anim. Sci. Biotechnol. 8:12

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## 421 Fermentation of phytochemicals to increase their biological activity

Alice König, Marion Dornmayr, Bettina Schwarzinger, Andreas Müller, Daniel Kofel and Julian Weghuber

Various phytochemicals are used as feed additives to improve meat quality characteristics, to support growth performance, or to prevent diseases in farm animals. In order to exert any biological effect, the bioactive compounds must be bio-available. However, glycosylation of phytochemicals often inhibits an efficient absorption and limits the beneficial effects in living animals. The release of bioactive compounds is a promising approach to improve bioavailability and health-promoting effects. Bacterial enzymes are known to cleave glycosidic linkages in plant-derived substances. Thus, we focus on fermentation to remove glycosidic units and to liberate bioactive metabolites. The idea behind is to increase the bio-efficacy of selected plant extracts by means of microbial cleavage.

An HPLC (high performance liquid chromatography) method was established to quantitate compounds of interest in selected plant extracts. Small scale-fermentation experiments were performed to test different probiotic strains for their ability to hydrolyze extract components. Therefore, the extract composition was analyzed before and after biotransformation to determine differences. Commercially available enzymes were used as positive control.

We were able to quantitate the main flavonoids in selected plant extracts using HPLC. Moreover, some lactic acid bacteria were able to cleave flavonoid-glycosides and increased the amount of aglycons in extract samples. These preliminary results are promising but we still want to determine, whether the released metabolites have improved health-promoting effects compared to non-fermented extracts. Therefore, molecular mechanisms affected by the relevant bioactive compounds will be investigated in cell culture experiments. We will study the impact on cellular stress and inflammation in order to determine, whether fermentation increases the antioxidant as well as anti-inflammatory activity of the used plant extracts. The results of this project will contribute to the evaluation of fermentation of phytochemicals regarding feasibility and efficiency for use in the feed industry.

### Author information

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Experience with cell culture assays; double-blind randomized clinical study

## 422 Fumonisin carboxylesterase FumD (FUMzyme®) counteracts the negative effects of fumonisin contaminated feed in weaning piglets in a 42-day utility study

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Fumonisin (FBs) are secondary metabolites predominately produced by *Fusarium verticillioides*. Fumonisin B1 (FB1), the most abundant representative, is known to be hepatotoxic, nephrotoxic and immunosuppressive. Exposure to FB1 is associated with porcine pulmonary edema and equine leukoencephalomalacia among others.

FBs are resistant to physical and chemical treatments. However, gastrointestinal degradation by using specific enzymes as feed additives is a promising strategy for detoxification of FBs in feedstuffs. We previously isolated the fumonisin degrading carboxylesterase FumD from *Sphingopyxis* sp. MTA144. This enzyme catalyzes the degradation of FB1 to hydrolyzed FB1 (HFB1), which shows strongly reduced toxicity compared to its parent compound. FUMzyme®, the commercial application of FumD, represents the first available recombinant mycotoxin degrading enzyme that is authorized as a feed additive in the European Union.

Here, we investigated the efficacy of FUMzyme® to degrade dietary FBs in the gastrointestinal tract of pigs in a 42-day feeding trial conducted at the Colorado Quality Research, Inc. (United States). A total of 150 pigs were randomly assigned to five treatment groups (n = 30, 10 pens per treatment, 3 either female or male pigs per pen). The negative control group T01 group received uncontaminated feed. The positive control group (T02) received feed contaminated with a total of ~6 mg/kg FBs. The three FUMzyme® treatment groups received feed contaminated with a total of ~6 mg/kg FBs and supplemented with 15 U/kg (T03), 30 U/kg (T04) or 45 U/kg (T05) FUMzyme®. To assess the efficacy of the enzyme, the Sa/So ratio in serum along with fecal contents of FB1 and HFB1 on day 21 and on day 42 were considered the pivotal parameters. Analysis was performed by validated LC-MS/MS methods. The pen was considered the experimental unit.

Compared to the positive control group T02, the Sa/So ratio was significantly decreased in all FUMzyme® treatment groups, indicating a significantly reduced toxicity. Furthermore, FB1 concentrations in feces were significantly reduced and concomitantly HFB1 concentrations were significantly increased in all FUMzyme® treated groups compared to group T02.

In conclusion, FUMzyme® degraded FBs in the gastrointestinal tract of pigs, thereby counteracting the toxic effects of fumonisin contaminated feed.

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## 423 Toxicological assessment of phytochemicals in *C. elegans*

Georg Sandner; Peter Lanzerstorfer; Johannes Pitsch; Bianca Mascher; Tobias Aumiller; Julian Weghuber

Essential oils (EOs) have attracted increased interest for different applications such as food preservatives, feed additives and ingredients in cosmetics. Due to their reported variable composition of components, they might be acutely toxic to humans and animals in small amounts. Despite the necessity, rigorous toxicity testing in terms of safety evaluation has not been reported so far, especially using alternatives to animal models. The replacement of animal trials is not only driven by ethical concerns but also the low throughput and high costs of critical parameters. Currently, many industries (e.g., the chemical, agricultural, food and cosmetics industries) are facing this problem due to the large and increasing number of chemicals, toxicants and additives in products and applications. Hence, nonanimal, higher throughput testing is of great interest and importance in toxicology.

For this purpose, a sensitive and suitable high-throughput test system using the nematode *C. elegans* was established. Selected EOs (rosemary, citrus, eucalyptus) were investigated regarding developmental and reproductive toxicity. Besides toxicological approaches, phytochemicals utilized as feed additives were further evaluated regarding their antioxidative capacity for improving animal health.

It was shown that even low concentrations of EOs exhibit toxic potential. *C. elegans* development and reproduction was already significantly inhibited at concentrations of 0.5% (wild-type) and 0.1% (bus-5) [v/v] of EO, respectively. Gene expression analysis revealed a significant upregulation of xenobiotic and oxidative stress genes such as *cyp-14a3*, *gst-4* and *sod-3*. Apart from toxicological approaches, several bioactive feed additives were also shown to provide antioxidative potential in *C. elegans*.

Detailed information about possible negative effects of phytochemical addition on animal and human health is often lacking. Within this regard, the toxic potential of phytochemicals might be underestimated, as natural compounds are not necessarily safer than other products. Thus, the toxic potential must be first investigated when phytochemicals are intended to be used for therapeutic aims or being incorporated feed and food products.

### Author information

Research associate and PhD student, extensive experience in *C. elegans*, gene expression, phytochemical feed additives

## 501 Nutrients content of fourth range salad leftover (SL) as feed ingredient for ruminant diets

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Fourth range salad leftover (SL) have good nutritional profiles and can serve as viable sources of protein, energy and fiber to meet the nutritional requirements of animals. However, SL nutritional potential for farm animals is not yet fully exploited. Thus, the aim of the present study was to evaluate the nutritional composition coupled with electronic nose odor profile of selected SL. Eighteen sample SL deriving from a plant producing fourth range salads have been collected during the summer season in two sites: at the production site (factory plant) and after transport and one-day storage (to mimic farm site). At the arrival at the lab, each sample was analysed using a portable electronic nose 3 (PEN 3) model e-nose from Airsense Analytics GmbH (Schwerin, Germany) in order to obtain an odour fingerprint. The same samples were then analyzed for pH, dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin. Data obtained were analysed using IBM SPSS Statistics version 21 software (SPSS Inc.). Odour profile of different samples indicated a clear distinction between the two sites of collection. Fourth range SL at the production site (factory plant) and after transport and one-day storage (to mimic farm site), have shown pH value of 6.0 and 6.4, respectively. For the same material nutrient composition was comparable to that of common fresh grass: the overall mean of CP content was 210 g kg<sup>-1</sup> DM, while average NDF, ADF and ADL contents were 360, 238, and 74 g kg<sup>-1</sup> DM respectively. The main difference between fresh grass and SL was for DM content, that was lower (5.8±0.8 g·kg<sup>-1</sup>) in SL samples compared to literature grass values. Accordingly, it can be concluded that fourth range SL has some potential as feed ingredient for ruminant's diets, even though a full verification of their potential need further deep investigation.

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## 502 Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on seaweed or selenium enriched substrates (note 1)

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This study evaluated the effect of inclusion of brown algae (*Ascophyllum nodosum*) or sodium selenite in Black soldier fly (BSF) growing substrate on the nutritional composition of the deriving prepupae. The larvae were reared on three different substrates: i) Gainesville diet used as control substrate (CTR) and ii) *Ascophyllum nodosum* diet (AN30%) with 30% substitution of the alfalfa meal with the brown algae iii) Selenium diet (Se) with Gainesville diet enriched with 0.3 mg·Kg<sup>-1</sup> of selenium in the form of sodium selenite. Eggs collected from a stable colony of BSF were placed for hatching on three experimental substrates CTR, AN30% and Se. All experiments were carried out under dark condition, at 25°C with 70% relative humidity, and repeated for three consecutive generations. Both rearing substrate and deriving prepupae were analyzed for proximate and fatty acid composition. Results were expressed on dry matter basis (DM). Total biomass produced, starting from 300 young larvae under different substrates were 20.9, 23.4, 20.7g pupae for CTR, AN30% and Se dietary groups, respectively. The mean protein content was 536 g·kg<sup>-1</sup> DM in CTR, 446 g·kg<sup>-1</sup> DM in AN30%, and 351 g·kg<sup>-1</sup> DM in Se group. Considering fatty acid composition, CTR, AN30%, and Se prepupae contained a high level of saturated fatty acids, namely: 74.8±2.7%, 83.0±0.5%, and 85.5±0.7% of total lipids, respectively. The inclusion of seaweed in the rearing substrate did not affect the fatty acid profile in the AN30% prepupae compared to CTR and Se dietary groups. From the obtained results it can be suggested that both the seaweed *Ascophyllum nodosum* and sodium selenite inclusion affect the BSF larvae composition in term of crude protein, with minor effect on lipid content and FA profile. However, further investigation are required for understanding the effect of the composition of the feeding media on the nutritional value of deriving larvae in order to produce BSF meal suitable for specific feed or food purposes.

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## 503 Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on seaweed or selenium enriched substrates (note 2)

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This study evaluated the effect of inclusion of brown algae (*Ascophyllum nodosum*) or sodium selenite in Black soldier fly (BSF) growing substrate on the nutritional composition of the deriving prepupae. The larvae were reared on three different substrates: i) Gainesville diet used as control substrate (CTR) and ii) *Ascophyllum nodosum* diet (AN30%) with 30% substitution of the alfalfa meal with the brown algae iii) Selenium diet (Se) with Gainesville diet enriched with 0.3 mg·Kg<sup>-1</sup> of selenium in the form of sodium selenite. Eggs collected from a stable colony of BSF were placed for hatching on three experimental substrates CTR, AN30% and Se. All experiments were carried out under dark condition, at 25°C with 70% relative humidity, and repeated for three consecutive generations. Both rearing substrate and deriving prepupae were analyzed for selenium concentrations and selenium species. Results were expressed on dry matter basis (DM). The mean total selenium concentration for prepupae in each treatment group, was: 0.41mg·kg<sup>-1</sup> in CTR, 0.21 mg·kg<sup>-1</sup> in AN30%, and 1.17 mg·kg<sup>-1</sup> in Se group. From the selenium speciation analyses the highest level of selenium selenite was found in the Se prepupae group, with concentration of 0.55 ± 0.02 mg·kg<sup>-1</sup> compared to the AN30% and CTR prepupae with 0.07 ± 0.01 mg·kg<sup>-1</sup> and 0.13 ± 0.03 mg·kg<sup>-1</sup>, respectively. From the obtained results it can be proposed that both the seaweed *Ascophyllum nodosum* and sodium selenite inclusion affect the BSF larvae total Se and selenium species. Specifically, in the present study total Se and selenium species ranked as follow Selenium group > CRT group > AN30%. However, further investigation are required for understanding the effect of the composition of the feeding media on the nutritional value of deriving larvae in order to produce BSF meal optimized for specific feed or food purposes.

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## 504 Emerging risks associated with "Novel feeds": Are we prepared for future safety challenges?

Michele Ardizzone<sup>1</sup>, Angelo Maggiore<sup>2</sup>, Georgia Gkrintzali<sup>2</sup>, Giovanni Savoini<sup>3</sup>, Ana Afonso<sup>1</sup>

The foundation of our current legislation on food and feed safety in Europe has evolved over the last two decades, mostly in response to food/feed crises. Bovine Spongiform Encephalopathy and Dioxins, both linked to animal feed, highlighted the need to safeguard human and animal health through improved regulatory safety standards, at national and European level. Continuous population growth and climate change will exacerbate the depletion of resources, which occurs at a rate exceeding the Earth's capacity to renew them. Growing demands for protein and energy sources will require the use of a large amount of natural resources in the feed sector, which is already under great pressure. This "protein/energy challenge" is nudging risk assessors and managers, industry, academia, and farmers to explore the benefits of alternative and more sustainable feed sources. More emphasis should be put on the development of a sustainable food and feed sector. Proposals have been made to move toward feed uses of "new sources of feed from the food industries, biofuel industries and industrial processes and new types of ingredients such as processed animal proteins derived from farmed insects, marine resources and aquatic plants". In the transition to sustainability, it will be crucial to identify emerging risks associated with these "novel feeds", new hazards, exposure pathways or knowledge gaps, and to prioritize future work to ensure the continued protection of humans, animals and the environment under the "One Health" approach. While a complex EU regulatory frame that determines how to assess a large variety of potential hazards in feed commodities is in place, a paradigm shift is needed for "novel feeds". In this respect, EFSA endeavours to ensure preparedness to future risk assessment, food/feed safety and environmental challenges. Harmonised activities in partnership with a broad group of stakeholders is important for the development of a modern feed risk assessment strategy that will address future needs, including a more holistic assessment of "novel feeds". Therefore, EFSA's work involves the mapping of "novel feed" sources and analysis of drivers, with the aim of identifying emerging risks associated to "novel feeds".

### Author information

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## 601 In situ ruminal degradability of dry matter and organic matter of extruded hempseed cake

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The aim of the study was to evaluate the influence of extrusion processing of hempseed cake (HSC) on ruminal degradability of dry matter (DM) and organic matter (OM). A 3x3 experimental setup was used, varying two extrusion parameters: moisture of the material in extruder barrel (16, 20 and 24%) and temperature along the barrel (90, 110 and 130 °C). Total of 9 extruded samples was produced. The ruminal disappearance of DM and OM was measured using the in situ nylon bag technique, where the test feedstuffs were subject to ruminal incubation in four rumen fistulated dry cows with rumen cannulae of 10.5 cm die opening. Disappearance of DM and OM from the test feeds from the rumen was measured at 0, 3, 6, 9, 16, 24 and 48 h. The exponential model of Ørskov and McDonald (1979) was used to calculate degradation kinetics. The effective degradability of DM (EDDM) and OM (EDOM) was calculated at outflow rate of 0.06 h<sup>-1</sup>. In untreated HSC determined EDDM and EDOM were 35.74 and 34.76%, respectively. The least significant influence of extrusion processing on both degradability parameters was observed after the treatment on 90 °C. An increase of extrusion processing temperature to 130 °C led to significant reduction of EDDM and EDOM. The influence of moisture content during extrusion was also observed - the lowest influence on both parameters was determined for 16% and highest for 24% moisture content. Obtained results indicate that high moisture (24%) and high temperature (130 °C) during extrusion processing produce hempseed cake with lowest EDDM and EDOM which were 19.27 and 18.66%, respectively. The determined results are important because based on the effective degradability of DM, as well as of other nutrients, it is possible to predict their passage into the small intestine. Obtained results confirm that extrusion processing can be successfully used to increase a passage of DM as well as of OM to small intestine of ruminants.

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## 602 Harmonization of methods of sampling and analysis of feed – Activities of CEN/TC 327

Charlotte Mosies<sup>1</sup>, Katarzyna Sobiech-Matura<sup>2</sup> and Siebren van Tuinen<sup>3</sup>

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission. Currently, EC SANTE and CEN/TC 327 are discussing the content of a new standardization request concerning analytical methods for animal feeding stuffs. At the time of the 2020 conference, it is expected details can be shared. In May 2018, CEN/TC 327/WG 2 "Composition" has been made dormant, to be awakened once there are documents to be reviewed or developed in their scope. Currently, CEN/TC 327/WG 6 "Radioactivity measurements" is finalizing a CEN standard with a method for the - Determination of the radionuclides <sup>131</sup>I, <sup>134</sup>Cs and <sup>137</sup>Cs in feed with gamma ray spectroscopy (European Standard EN 17462:2021 ' Animal feeding stuffs: Methods of sampling and analysis — Determination of the radionuclides iodine-131, caesium-134 and caesium-137 in feed', expected to be published mid 2021). One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

### Author information

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## 603 Harmonization of methods of analysis for organic contaminants in feed – Activities of CEN/TC 327/WG 1

Merel Wagner<sup>1</sup>, Hildburg Fry<sup>3</sup>, Florence Lacoste<sup>4</sup>, Ruud Peters<sup>2</sup> and Jacob de Jong<sup>2</sup>

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One its technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission. Currently, EC SANTE and CEN/TC 327 are discussing the content of a new standardization request concerning analytical methods for animal feeding stuffs. At the time of the 2021 conference, it is expected details can be shared.

CEN/TC 327/WG 1 "Organic contaminants" is currently drafting a CEN standard with methods for the analysis of saturated hydrocarbons in vegetable oil by GC-FID (FprEN 17517). One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of the aforementioned method is in its final stage of progress. For the method currently being drafted, a dedicated poster will be presented by the project leaders.

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## 604 Harmonization of methods of analysis for additives and drugs in feed – Activities of CEN/TC 327/WG 3

Charlotte Mosies<sup>1</sup>, Jaroslava Petrová<sup>2</sup>, Jens Schönherr<sup>3</sup>, Atte von Wright<sup>4</sup> and Christoph von Holst<sup>5</sup>

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission.

Currently, EC SANTE and CEN/TC 327 are discussing the content of a new standardization request concerning analytical methods for animal feeding stuffs. At the time of the 2020 conference, it is expected details can be shared.

Currently CEN/TC 327/WG 3 "Feed additives and drugs" is drafting methods for the determination of:

- Bacteria through PFGE typing
- Carotenoids by RP-HPLC-UV
- Vitamin A, D and E content by SPE clean-up and HPLC
- Various probiotics (revision of EN 1578x-series).

For the first item, an European Standard (EN) is expected to be published in 2022, 'Animal feeding stuffs: Methods of analysis — PFGE typing of Lactobacilli, Pediococci, Enterococci and Bacilli in animal feeds'.

The other items are currently in the stage of finalizing an European Standard, meaning publications in 2021 for the following two (new) standards:

- EN 17550 'Animal feeding stuffs: Methods of sampling and analysis – Determination of carotenoids in animal compound feed and premixtures by High performance liquid chromatography – UV detection (HPLC-UV)' and
- EN 17547 'Animal feeding stuffs: Methods of sampling and analysis – Determination of vitamin A, E and D content – Method using solid phase extraction (SPE) clean-up and high-performance liquid chromatography (HPLC).

And the publication in 2021 for the following revised standards:

- EN 15784 'Animal feeding stuffs: Methods of sampling and analysis — Isolation and enumeration of Bacillus spp. used as feed additive';

## 604 Harmonization of methods of analysis for additives and drugs in feed – Activities of CEN/TC 327/WG 3

Continued

- EN 15786 'Animal feeding stuffs: Methods of sampling and analysis — Isolation and enumeration of *Pediococcus* spp. used as feed additive';
- EN 15787 'Animal feeding stuffs: Methods of sampling and analysis — Isolation and enumeration of *Lactobacillus* spp. used as feed additive';
- EN 15788 'Animal feeding stuffs: Methods of sampling and analysis — Isolation and enumeration of *Enterococcus* (*E.faecium*) spp. used as feed additive — Complementary element';
- EN 15789 'Animal feeding stuffs: Methods of sampling and analysis — Isolation and enumeration of *Saccharomyces cerevisiae* used as feed additive'.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

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## 605 Harmonization of methods of analysis for elements in feed and their chemical species – Activities of CEN/TC 327/WG 4

Merel Wagner<sup>1</sup> and Jens Sloth<sup>2</sup>

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of its technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission. Currently, EC SANTE and CEN/TC 327 are discussing the content of a new standardization request concerning analytical methods for animal feeding stuffs. At the time of the 2021 conference, it is expected details can be shared.

CEN/TC 327/WG 4 "Elements and their chemical species" currently plans to draft a method for the determination of fluoride content after HAT by ISE (revision of EN 16279:2012; new work item proposal under preparation).

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. When/If more details on the new standardization request concerning analytical methods for animal feeding stuffs are available at the time of the 2021 conference, (a) dedicated poster(s) will be presented by the project leaders.

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## 606 Harmonization of methods of analysis for natural toxins in feed – Activities of CEN/TC 327/WG 5

Suzanne de Jonge<sup>1</sup>, Dorina Bodi<sup>2</sup>, Patrick Mulder<sup>3</sup> and Hans Mol<sup>3</sup>

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission. Currently, EC SANTE and CEN/TC 327 are discussing the content of a new standardization request concerning analytical methods for animal feeding stuffs. At the time of the 2021 conference, it is expected that details can be shared.

Currently CEN/TC 327/WG 5 "Natural toxins" is drafting methods for the determination of:

- Free gossypol by LC-MS/MS;
- Intact glucosinolates by LC-MS/MS;
- Pyrrolizidine alkaloids by LC-MS/MS.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

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## 607 Analytical assessment of 930 field samples shows a large variation on nutritional and oxidative characteristics of fats and oils

Erwin Witters, Karen Bierinckx, Alexandra L. Wealleans, Mauro di Benedetto

Today, nutritionists risk decreased animal performance due to inaccurately allocated dietary energy values and induced metabolic stress by oxidised lipids. 930 fat and oil samples collected from the entire EMENA region were scrutinized through a portfolio of 10 chemical characteristics (Lipid Evaluation Test, LET). Two qualitative parameters, peroxide value and thiobarbituric acid value, estimate the degree of lipid oxidation. Measurements in Oxidative Stability Instrument (OSI) estimate of sensitivity for future oxidation and recovery of antioxidants, including tocopherols is done. Three nutritional parameters are evaluated, the free fatty acids content, the ratio of unsaturated over saturated fatty acids and the level of short chain fatty acids. These parameters are used in the Wiseman model to estimate the dietary energy contribution of lipids in feed for broilers and pigs. Three energy-diluting factors: moisture (M), impurities (I) and unsaponifiables (U) are incorporated. Almost 66% of the samples is represented by 5 groups: soybean oil (SO), animal fat (AF), acid oils (AO), sunflower oil (SFO) and poultry oil (PO). The assessment showed that in 64% of the cases signs of oxidation were detected. Initial oxidation (PV<5meq. kg-1) was determined in SO at 58%, AF at 31%, AO at 41% and PO at 7% except for SFO where 75% of the samples showed strong primary oxidation (PV>10 meq.kg-1). Analysis of the dataset confirmed large variation in dietary energy up to 40% between identical fat sources (AO). When the inclusion of MIU factor is accounted for, there is even more excessive variation (310% AF) on nutritional value.

The analytical assessment gives insight in the large variation present between different lipid sources. The compiled data helps to fully understand the huge variability in nutritional and oxidative quality of fats and oils.

### Author information

Erwin Witters is a research professional with more than 20 years of experience. He heads Kemin's customer laboratory services section with focus on feed preservation and animal health.

## 608 Microbiological quality of feed

Manuela Zadavec, Nicolas Pradervand, Igor Ujčić Vrhovnik

In most countries, microbiological quality of feeds is sadly a much-underrated aspect of official feed control. Indeed, monitoring the microbiological quality of feeds (i) downgrades the poor quality feeds, (ii) stimulates mills to produce better quality feeds and (iii) enforces the prominence of high quality feeds on the market. Those high quality feeds, when vastly available on the markets, will then fuel better performances of farm animals and thus sustain more successful farmers. Moreover, monitoring microbial feed quality also allows shedding the light on mills with systemic bad hygiene, unsatisfactory storing environment or working with low-grade raw materials. On the latter, the microbiological feed quality analysis enables to pinpoint dangerous species of moulds (mycotoxins producers), that are often not covered by the traditional feed safety spectrum. Those moulds could originate either from disease-stricken plant material (e.g. *Fusarium* spp) or from inadequate storing conditions (e.g. *Aspergillus* spp and *Penicillium* spp). The same is true for the aerobic mesophilic bacterial flora of those feeds, which can be either associated with epiphytic bacteria "from the field" or from the flora that develops during storage. Total yeasts counts are also analyzed.

Feed quality is assessed through a single VDLUFA method used for decades by the laboratory members of the European Feed Microbiology Organization (EFMO). This involves (i) counting the bacteria, moulds and yeasts present in the samples, (ii) classifying them into 7 functional categories and (iii) comparing the obtained concentrations of categories to elaborate „normal“ values for each type of matrices and taking into account the types of feeding animals.

The aim was to gather the results of microbiological feed quality of three countries and to make a survey of the monitoring over a year. 125 samples from Croatia, 46 from Slovenia and 130 from Switzerland are used in this study. The results give an overview of the advantages of monitoring the microbiological quality of feeds for both governmental authorities, producers and consumers alike and potentially enlighten new aspects of the risk assessment of certain types of feeds or their raw materials

### Author information

All authors work in official laboratories of their states on the field of feed hygiene.

## 609 (Bio)technological processing of rapeseed cake for broiler nutrition

F. Goodarzi Boroogeni<sup>1</sup>, K. Kozłowski<sup>2</sup>, A. Drażbo<sup>2</sup>, and J. Zentek<sup>1</sup>

This study evaluated the effect of partial substitution of soybean meal (SBM) in broiler diets with (bio)technologically processed rapeseed cake on growth performance and nutrients digestibility. Rapeseed cake (RC) was fermented without (WR) or with enzymes (ENR, phytase, pectinase and  $\beta$ -glucanase). A standard corn-SBM diet was produced as control group (CON). Three experimental diets were produced using untreated (NRC) and fermented (WR and ENR) RC products as substitutes for SBM at 15% inclusion level. Fifth diet (IFE) was produced using 15% untreated RC and in-feed inclusion of phytase, pectinase and  $\beta$ -glucanase (RONOZYME HiPhos and VP, DSM). Diets were isonitrogenous and isocaloric. Performance variables were recorded during starter (d 1-21) and grower (d 22-35) periods as well as at the end of the experiment (d 35). Apparent ileal digestibility (AID) of nutrients was determined at d 35. Data were subjected to ANOVA using the GLM procedure. Body weight gain and feed intake of all the experimental groups were similar during different experimental periods ( $P > 0.05$ ). At the end of the starter period and entire experiment, feed conversion ratio (FCR) of broilers fed the IFE diet was better ( $P \leq 0.05$ ) than those receiving the CON or NRC diet. During the starter period, broilers in the ENR group demonstrated better FCR than those in the CON or NRC group but at the end of the experiment, birds fed the ENR diet showed better FCR compared with those fed the NRC diet ( $P \leq 0.05$ ). AID of protein and fat was identical for all the experimental groups ( $P > 0.05$ ). ENR and IFE diets had higher AID of P compared with the NRC, WR or CON diet ( $P \leq 0.05$ ). Birds in the IFE group displayed higher AID of Ca compared with those in the NRC or WR group, with those in the NRC group presenting the lowest AID of Ca ( $P \leq 0.05$ ). In conclusion, partial replacement of SBM with enzymatically fermented RC or adding enzymes to diets containing 15% untreated RC can improve AID of Ca and P as well as feed efficiency in broiler nutrition.

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## 610 Different heat treatments and soybean varieties and their effect on gut microbiota in broilers

Florian Hemetsberger, Dipl.-Ing., Wolfgang Kneifel, Univ.Prof. Dipl.-Ing. Dr.nat.techn., Karl Schedle, Priv.-Doz. Dipl.-Ing. Dr., Konrad Domig, Univ. Prof. Dipl.-Ing. Dr.nat.techn.

Soybeans are an important part of diets for monogastric animals because of its high protein content and digestibility. However, soybeans require a heat treatment before feeding to eliminate the activity of anti-nutritional factors and guarantee high digestibility. Although a heating process is needed, an excess of applied heat increases loss of nutrients such as amino acids and promote the formation of compounds such as Maillard reaction products. These heat-induced changes of the diet might have an effect on the intestinal microbiota of the animals. To evaluate the effect of heat damaged feed ingredients on the gut microbiota, soybean cakes were processed with two different heat intensities and fed to broiler chickens. Soybeans of two different varieties were partially de-oiled and autoclaved at 110° or 120°C, resulting in four experimental treatments. 336 day-old mixed-sex chicks (Ross 308) were allocated into 24 pens, resulting in six replicates per treatment. Chickens were fed a diet including 30% of the different soybean cakes for 36 days. On day 36 animals were slaughtered and digesta of ileum and caecum was collected for microbial analysis. Total DNA of chyme was extracted, prepared and sequenced using the Illumina MiSeq system. Microbial community of ileum and caecum showed significant differences considering alpha and beta diversity parameters ( $p < 0.001$ ). Firmicutes represented the most abundant phylum in ileum and caecum (96.0 and 90.9%). The most abundant bacterial family in the ileum was Lactobacillaceae, while Peptostreptococcaceae being the most abundant family in the caecum. Heat treatment and soybean variety showed little effect on alpha diversity of the microbiota. In the caecum higher treatment temperatures led to a higher number of observed operational taxonomic units ( $p < 0.05$ ). No differences regarding abundance of any phylum caused by soybean variety or processing intensity was observed. On family level high processing temperatures led to an increased abundance of Clostridiaceae ( $p < 0.05$ ) in the ileum and a decreased abundance of Lachnospiraceae ( $p < 0.01$ ) and Streptococcaceae ( $p < 0.05$ ) in the caecum.

Microbial community in the gut of broiler chickens showed distinctions between gut sections. Though, temperature as well as soybean variety showed limited effects on the composition of gut microbiota.

### Author information

Phd student in animal nutrition

## 611 Importance of homogeneity and carryover monitoring in the feed mill

Olivera Đuragić, Radmilo Čolović, Vojislav Banjac, Bojana Kokić, Danka Dragojlović, Sanja Popović, Strahinja Vidosavljević

Feed production is considered as an important part of the food chain. It is necessary to meet the complex nutritional requirements for different species and categories of animals. A large number of ingredients, as well as their share, complicates the whole process, due to their different physical and chemical properties. It is important to achieve the homogeneity across the whole production line avoiding the accumulation of the potentially toxic components in the critical control points of the process line (Djuragic et al., 2017). This study was carried out to determine and monitor the critical points of the feed production line. The samples were taken from the mixer, elevator, chain conveyor, bins and bags to determine homogeneity and level of carry-over over. Determination of homogeneity and carry over level was done by Microtracer® method (Micro Tracers, Inc., San Francisco, CA 94124). Results showed that homogeneity was achieved at the beginning of the process, in the mixer itself. The values of the coefficients of variation and/or probability P, according to the criteria for homogeneity, are in the range that declares a good level of homogeneity (CV = 8.98% and probability P = 21.68%). Slightly higher values (CV = 9.07% and P = 18.51%) were obtained in the samples taken at the outlet of the bin but were still within the limits for good homogeneity. Samples taken after the elevator, at the exit of the chain conveyor, indicate mixture segregation and no longer meets the criteria for homogeneity (P = 0.01% and P = 0). The samples were taken after the first cleaning batch contained the largest number of particles, while the number of particles in subsequent samples decreased rapidly. The highest level of carryover (0.7% and 0.8%) was recorded at the exit of the elevator and chain conveyor, the possible caused by the backlog of materials in these places (elevator buckets, chain conveyor bottom). Although the transmission level is within the limits considered to be non-critical (up to 4%), depending on the further production process and equipment condition, the transmission level may be far higher.

### Author information

Senior researcher, specialized in the technology of animal feed production, working accuracy of the process (homogeneity of compound feeds, cross-contamination and carry over)

## 612 Assessment of feed storage contamination by fungi and producing mycotoxin on animal feed

Kgomotso Galian Setsetse<sup>1</sup>, Mulunda Mwanza<sup>2</sup>

**Introduction:** The improper storage system of feed is a major factor influencing the presence of fungi and mycotoxin contamination. Hence, two types of feed storage systems were studied, just about 41.7% of emerging farms used open storage system and 58.3% used closed storage systems whereby animal feeds were preserved in bags or dustbin, whilst feed commercial suppliers mainly used closed storage.

**Material and Methods:** A total of 100 animal feed samples were collected randomly from emerging farms (40) and commercial feed supply storages (60) around Ngaka Modiri Molema, North West, were classified as either open or closed feed storage. Aflatoxin B1, B2, G1 and G2 were Isolated and confirmed using the molecular techniques and Polymerase Chain Reaction (PCR). The mycotoxins extraction, determination, and quantification were done using the ELISA and HPLC and TLC methods.

**Results:** *A. flavus* the B-type and *A. parasiticus* that were isolated in this study produce both B-type and G-type Afs (AFG1 and AFG2) with high mean concentration 632.03 - 618.91 ppb, Ochratoxin A (387 ppb and 575 ppb), fumonisin (B1, B2) with mean value of 525.5 ppb and 193.67 ppb conducted from closed storages from emerging farm and feed commercial suppliers, respectively. These means were beyond the acceptable regulated levels in South Africa and in the world. Zearalenone revealed low mean concentrations of (2.20 – 2.64 ppb and 0.16 ppb) in emerging farm storages (Closed and open) and commercial feed suppliers closed storage, respectively.

**Conclusions:** Current data obtained revealed that closed commercial feed suppliers' storages were highly contaminated due to the prolonged storage of feed, lack of temperature regulation and ignorance, whilst the positive results obtained from samples from emerging farms (closed & open) could be due to poor storage facilities, improper handling conditions and transportation might be some of the factors contributing to feeding contamination. In addition, the study showed that there was a significant difference between closed and open storage regarding fungi and mycotoxin contamination.

**Keywords:** Feed storage, fungal and mycotoxin contamination, feed quality

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### 613 Determination of trypsin inhibitor activity in soybeans and derived products: a modified method of ISO 14902:2001 for higher throughput

DI Magdalena Wagner, Anna Krottenthaler Bsc, Dr. Elisabeth Reiter, DI Irmengard Strnad

Soybeans are widely used in human and animal nutrition due to their high protein content and valuable amino acid profile (Paulsen 2008). Raw soybeans contain anti-nutritional substances including trypsin inhibitors (TI) which lower the activity of the digestive enzymes trypsin and chymotrypsin and reduce the digestibility of main ingredients, minerals and vitamins in mammalian organisms (Brugger et al. 2019). The trypsin inhibitor activity (TIA) has been established as an important quality criterion for soybeans and derived products that are used in animal feed. The uptake of nutritive substances in the digestive system can be improved by heat treatment of raw soybeans (Sueiro et al. 2015). Several procedures to measure TIA have been developed where enzymatic assays are most widely used to determine whether the heating procedure was sufficient or not (Avilés-Gaxiola et al. 2018). The standard method ISO 14902 describes an enzymatic assay that works with high volumes of expensive reagents and is limited to a small number of samples due to inconvenient handling. Therefore, the aim of this study was to miniaturize the photometric assay to a microtiter scale. The outcome should be a simple protocol for convenient handling of a larger number of samples accompanied by lower costs for expensive chemicals. Many different experiments were carried out to develop a method with reproducible and comparable results to the standard method. These tests included changes of the incubation time, incubation temperature and the final test volume. Results show that comparable TIA levels can be measured by conducting the microtiter assay and the standard method.

#### Author information

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### 614 Specific density as parameter for separating feed ingredients

L.W.D. van Raamsdonk, B. Hedemann, J. Vliege, C.P.A.F. Smits, T.W. Prins

Specific density of materials is used in a range of disciplines, ranging from mineralogy to specific feed products. Especially the separation of the mineral fraction from the other ingredients in compound feeds is common procedure for the detection of animal proteins (Regulation (EC) 152/2009, Annex VI). Bone fragments, if present, will be collected in the (heavy) mineral fraction, allowing easy detection. At the other end of the range of different specific densities, muscle fibres and insect parts can be separated because of their relatively low specific density (Bremer et al., 2012; Veys and Baeten, 2018).

In principle, vegetable feed ingredients can be assumed to have a specific density larger than muscle fibres and insect parts and lower than minerals and bone fragments. The opportunity to separate these three major categories has been sufficiently demonstrated. However, differences among the diverse types of vegetable materials have never been explored. It is hypothesized that the parameter specific density can aid the identification of these materials.

The specific density was established for a range of single feed ingredients. In addition, experiments with mixtures of two ingredients have been carried out. Selected portions of material resulting from the separation experiments have been identified in order to confirm the effect of separation. The results show a diversity of specific densities of vegetable ingredients and opportunities for separation of different ingredients.

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## 615 Re-Evaluation of the protein content in maize varieties in Austria

Elisabeth Viktoria Reiter, Klemens Mechtler, Carmen Wilhelmer, Maximilian Lippl, Hans Felder, Oliver Alber, Irmengard Strnad

According to currently valid feed value tables maize kernels are described with an average protein content of 8.7 – 9 % in fresh matter. However, protein analyses in recent years obtained an average content of 7.8 % in fresh matter. The aim of the present project (Mais-XP, DaFNE: 101351) is to evaluate the protein content and the protein quality of the Austrian maize assortment in order to obtain more actual data. Therefore, during the project period (2018 – 2020), 480 protein analyses and approx. 220 amino acid analyses were performed annually.

Analyses of the Austria grain maize assortment from 2018 to 2020 resulted in an average protein content of 6,6 – 10,8 % per kg dry matter. The protein content of 10,2 % in dry matter (around 9 % in fresh matter) as laid down in feed value tables, was hardly achieved. With regard to on-farm-mixing of feed, an adapted feed value table is necessary to allow a more precise feed formulation. In addition, results are used for evaluation of nitrogen use efficiency, which is important for sustainable crop and animal production as well as for protection of climate and environment.

Results of maize kernel samples harvested in 2018 and 2019 indicate that early maturity groups have higher protein content than late maturity groups, whereas late maturity groups displayed higher protein yields than varieties of early maturity groups due to higher kernel yield. Furthermore, differences between the varieties in the protein and the amino acid content were observed.

### Author information

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## 616 Milk yield and milk composition of dairy cows fed pomegranate-peel extract

M. J. Abarghuei, Y. Rouzbehan, A. Z. M. Salem, M. J. Zamiri

An experiment was carried out to determine the effect of pomegranate peel extract (PPE) on dry matter intake (DMI), milk yield, milk protein, milk fat, and milk fatty acid profile of dairy cows. Four Holstein cows were used in a 4 x 4 Latin square design with 28-dperiods and 4 treatments: PPE0 (no extract), PPE400 (400 ml PPE/cow/d), PPE800 (800 ml PPE/cow/d) and PPE 1200 (1200 ml PE/cow/d). DMI, milk yield, milk protein, milk fat, and the milk fatty acid profile were measured. Determination of secondary metabolites of PPE showed its high content of aqueous fraction, total phenolics and total tannin with reasonable content of saponins. Milk yield, 4% FCM, milk fat and protein production (kg/d) and milk efficiency were increased by inclusion of PPE at 800 ml PPE/cow/d in the diet. When compared milk from cows that received no PPE, the dietary supplementation with PPE at 800 ml PPE/cow/d had lowered total saturated fatty acids, desirable  $\omega 6/\omega 3$  ratio and increased content of Eicosapentaenoic acid (EPA, C20:5) and Docosahexaenoic acid (DHA, C22:6) by inclusion of PPE at 800 ml PPE/cow/d in the diet. The results suggested that PPE supplementation at 800 ml PPE/cow/d in the diet had increased milk yield, 4% FCM, milk fat and protein production (kg/d) and milk efficiency, but lower total saturated fatty acid, desirable  $\omega 6/\omega 3$  ratio and higher content of EPA and DHA.

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